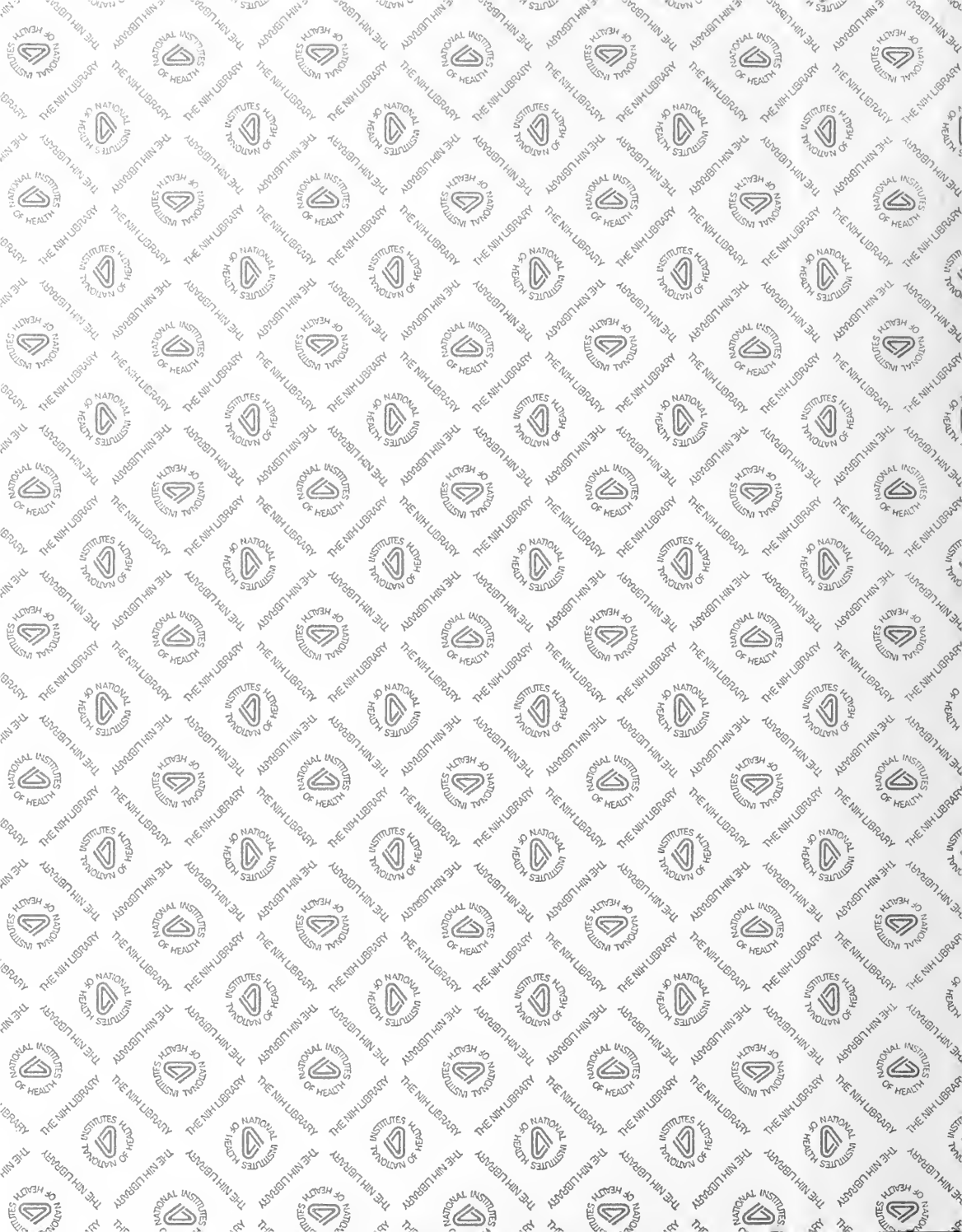
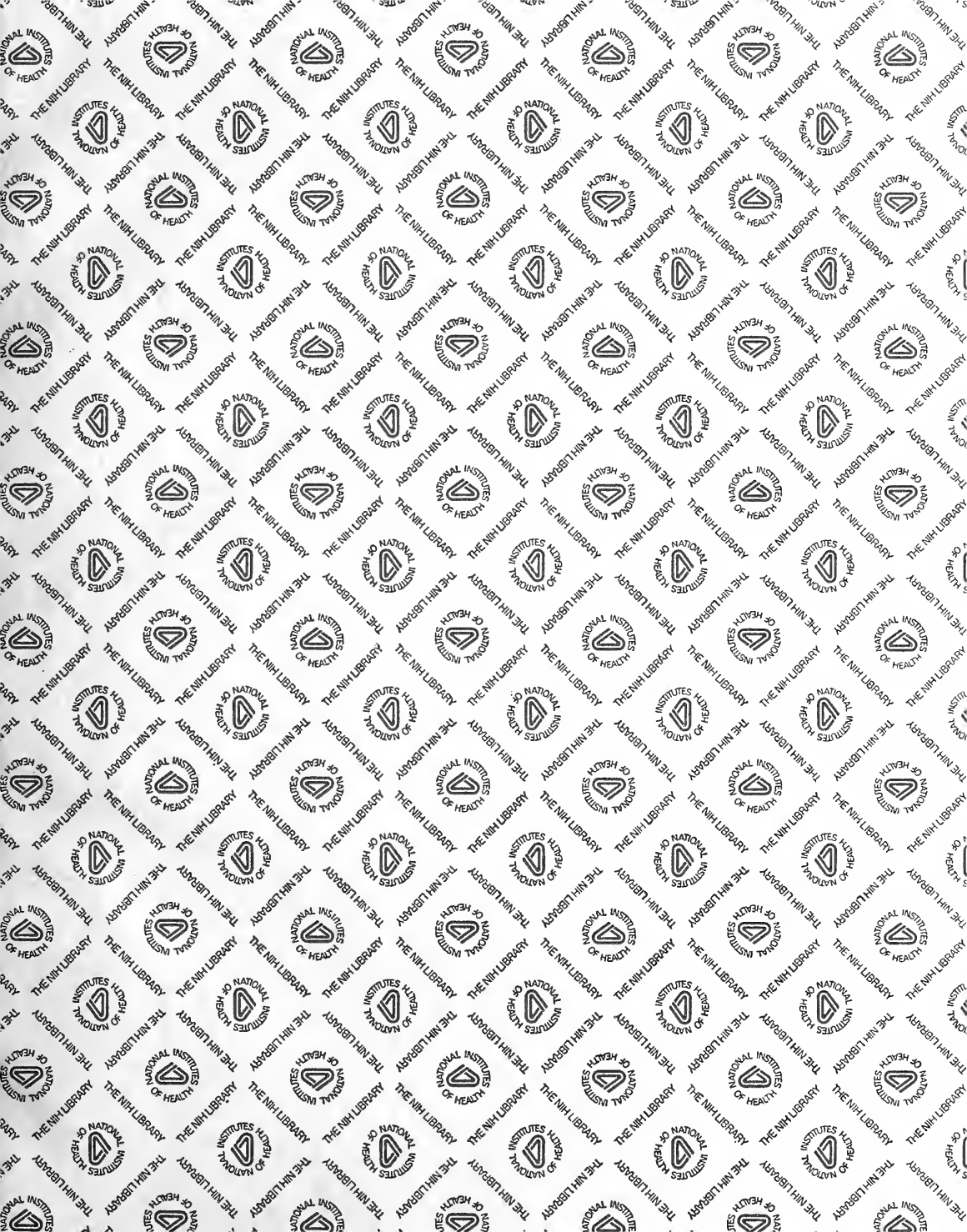


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<i>INTRAMURAL PROJECT SUMMARIES</i>		
National Cancer Institute Division of Cancer Prevention and Control		
'94 Annual Report		
<div data-bbox="461 973 725 1148" data-label="Image">A rectangular library stamp from the National Institutes of Health (NIH) Library. The text on the stamp reads: "NATIONAL INSTITUTES OF HEALTH" at the top, "NIH LIBRARY" below it, the date "JAN 25 1995" in the center, and "BUILDING 10" and "BETHESDA, MD 20892" at the bottom.</div>		

DCPC Intramural Annual Report — Summary

I - Director's Report

includes Organization Chart

II - Intramural Project Summaries

Z01 CN 00100-12 CPSB	Alpha-Tocopherol, Beta-Carotene Lung Cancer Prevention Study
Z01 CN 00101-12 CPSB	Human Studies of Diet and Nutrition
Z01 CN 00103-12 CPSB	Use of Isotretinoin in Prevention of Basal Cell Carcinoma
Z01 CN 00104-12 CPSB	NHANES I Epidemiologic Followup Survey: Chemoprevention/Nutrition Aspects
Z01 CN 00105-12 BB	Research in Cancer Screening and Statistical Methodology
Z01 CN 00106-12 BB	Studies in Cancer Screening
Z01 CN 00107-12 BB	Design and Analysis of Pharmacokinetic Studies of Selenium
Z01 CN 00112-11 CPSB	Nutrition Intervention Studies of Esophageal Cancer in Linxian, China
Z01 CN 00116-11 BB	Statistical Methodology Research
Z01 CN 00119-11 BB	Consultation on Clinical Trials and Other Studies
Z01 CN 00121-10 BB	Research in Biostatistical Methodology and Mathematical Modeling
Z01 CN 00142-10 BB	Cancer Control Objectives and Cancer Mortality Projections
Z01 CN 00143-10 CPSB	Continued Followup of the Breast Cancer Detection and Demonstration Project
Z01 CN 00146-06 CPSB	Nutritional Factors and Cancer in the Framingham Heart Study
Z01 CN 00147-06 CPSB	Nutritional Factors and Cancer in the Framingham Offspring Study
Z01 CN 00148-06 CPSB	Finland Studies of Nutrition and Cancer
Z01 CN 00149-06 CPSB	Yunnan Tin Miners Lung Cancer Studies
Z01 CN 00150-06 CPSB	Esophageal Cancer Genetics Studies
Z01 CN 00151-06 CPSB	A Dietary Intervention Study of the Recurrence of Large Bowel Adenomatous Polyps
Z01 CN 00153-05 CPSB	Evaluation of the Effects of a Fat-Modified Diet on Hormones During Adolescence
Z01 CN 00154-05 CPSB	Fels Early Nutrition and Growth Study
Z01 CN 00155-04 LNMR	A Mechanism for Carcinogen Resistance: Dietary Regulation of Carcinogen Efflux
Z01 CN 00156-04 LNMR	Nutritional Regulation of Carcinogens in Placenta-Related Cells
Z01 CN 00157-04 LNMR	The Effect of Proteins, Peptides, and Amino Acids on Carcinogenesis
Z01 CN 00158-04 LNMR	Nitric Oxide, Dietary Factors, and Signal Transduction
Z01 CN 00159-04 LNMR	Nutritional Modulation of Apoptosis: Regulation of Oncoproteins and Suppressors
Z01 CN 00160-04 LNMR	Nutritional Regulation of <i>Ras</i> Proto-Oncogene Activity
Z01 CN 00161-04 LNMR	Role of Fiber and Phytohormones in Cancer Prevention
Z01 CN 00162-04 LNMR	Effects of Vitamin A Nutriture and Synthetic Retinoids on Retinol Metabolism

Z01 CN 00163-03 LNMR	Mechanisms for Deranged Androgen Response in Prostate Cancer Cells
Z01 CN 00164-03 BPRB	The Molecular Mechanisms of Oncogene Action
Z01 CN 00165-03 BPRB	The Use of Transcriptional Factors as Targets and Agents for Chemoprevention
Z01 CN 00166-03 BPRB	Expression of TGF- β Isoforms in Human Lung Cancer Cells
Z01 CN 00167-03 BPRB	Cellular Differentiation in Normal and Neoplastic Respiratory Epithelium
Z01 CN 00168-03 BPRB	<i>CYP1A1</i> Gene Regulation and Human Cancer
Z01 CN 00169-03 BPRB	Rational Applications of Biomarkers in Clinical Trials
Z01 CN 00170-03 BPRB	Clinical Evaluation of New Intervention Agents
Z01 CN 00171-03 BPRB	Biological Regulation of Lung Cancer Growth
Z01 CN 00172-03 BPRB	Immunocytochemical Test for Early Lung Cancer Detection CRADA# 35516
Z01 CN 00173-03 BPRB	Identification of Peptide Growth Factors That Regulate Human Tumor Proliferation
Z01 CN 00175-03 BPRB	Post-Translational Processing Mechanisms in Tumor Cells
Z01 CN 00176-03 CPSB	Biologic Specimen Bank for Early Lung Cancer Markers in Chinese Tin Miners
Z01 CN 00177-03 BB	Research in Statistical Methodology and Consultation for Cancer Prevention
Z01 CN 00179-02 BPRB	Role of Transcription Factors in Breast Epithelial Cells
Z01 CN 00180-02 BPRB	Evaluation of Markers for the Early Detection of Breast Cancer
Z01 CN 00181-02 BPRB	The Molecular Genetics of Gynecologic Cancers
Z01 CN 00182-02 BPRB	Biochemistry of Peptides and Growth Factors in Lung Cancer
Z01 CN 00183-02 BPRB	Evaluation of Markers for the Early Detection of Lung Cancer
Z01 CN 00184-01 CPSB	Predagnostic Breast Cancer Serum Bank (Columbia, Missouri)
Z01 CN 00185-01 CPSB	Early Detection of Esophageal Cancer
Z01 CN 00186-01 CPSB	NCI-AARP Health Study
Z01 CN 00187-01 BB	Statistical Inference in Model Selection
Z01 CN 00188-01 BB	Statistical Inference in Stochastic Regression Models
Z01 CN 00189-01 LNMR	Dietary Regulation of Biochemical/Molecular Change in Carcinogen Resistant Cells
Z01 CN 00190-01 LNMR	Mechanisms of Diet and Chemoprevention in p53-Knockout Transgenic Mice
Z01 CN 00191-01 LNMR	Control of P-glycoprotein Function by Iron
Z01 CN 00192-01 BPRB	Growth of Human Tumor Cell Lines in Protein-Free Media
Z01 CN 00193-01 BPRB	Development of Molecular Markers for Early Detection of Epithelial Tumors
Z01 CN 00194-01 BPRB	Regulation of Differentiation During Lung Carcinogenesis
Z01 CN 00195-01 BB	Consultation in Biostatistical Methodology and Cancer Control
Z01 CN 00196-01 BB	Community Intervention Trial for Smoking Cessation (COMMIT)
Z01 CN 00197-01 BB	Brain Tumor Clinical Trials

DIRECTOR'S REPORT

This report describes the intramural research activities of the Division of Cancer Prevention and Control (DCPC), one of the five program divisions of the National Cancer Institute (NCI). The mission of the DCPC encompasses basic and applied research on cancer prevention, cancer control research, public health applications research including technology transfer, and cancer surveillance, all aimed at the overall goal of the NCI: to reduce the incidence, mortality, and morbidity of cancer. Intramural research is one of the foundation stones of both the National Cancer Institute and the Cancer Prevention and Control Program.

The DCPC conducts the full spectrum of research on prevention and control, from the earliest stages of hypothesis development through clinical studies and trials and through defined population studies, all leading to a program of demonstration and evaluation studies. The Division's activities include research on prevention, evaluation of screening and early detection regimens, research on cancer among special populations, and research on rehabilitation and continuing care. A major emphasis is cancer prevention and early detection research, with significant efforts (particularly in the intramural program) devoted to research on diet, nutrition, chemoprevention and biomarkers. As outlined below, the DCPC's intramural program is composed of three Branches and one Laboratory.

ORGANIZATION

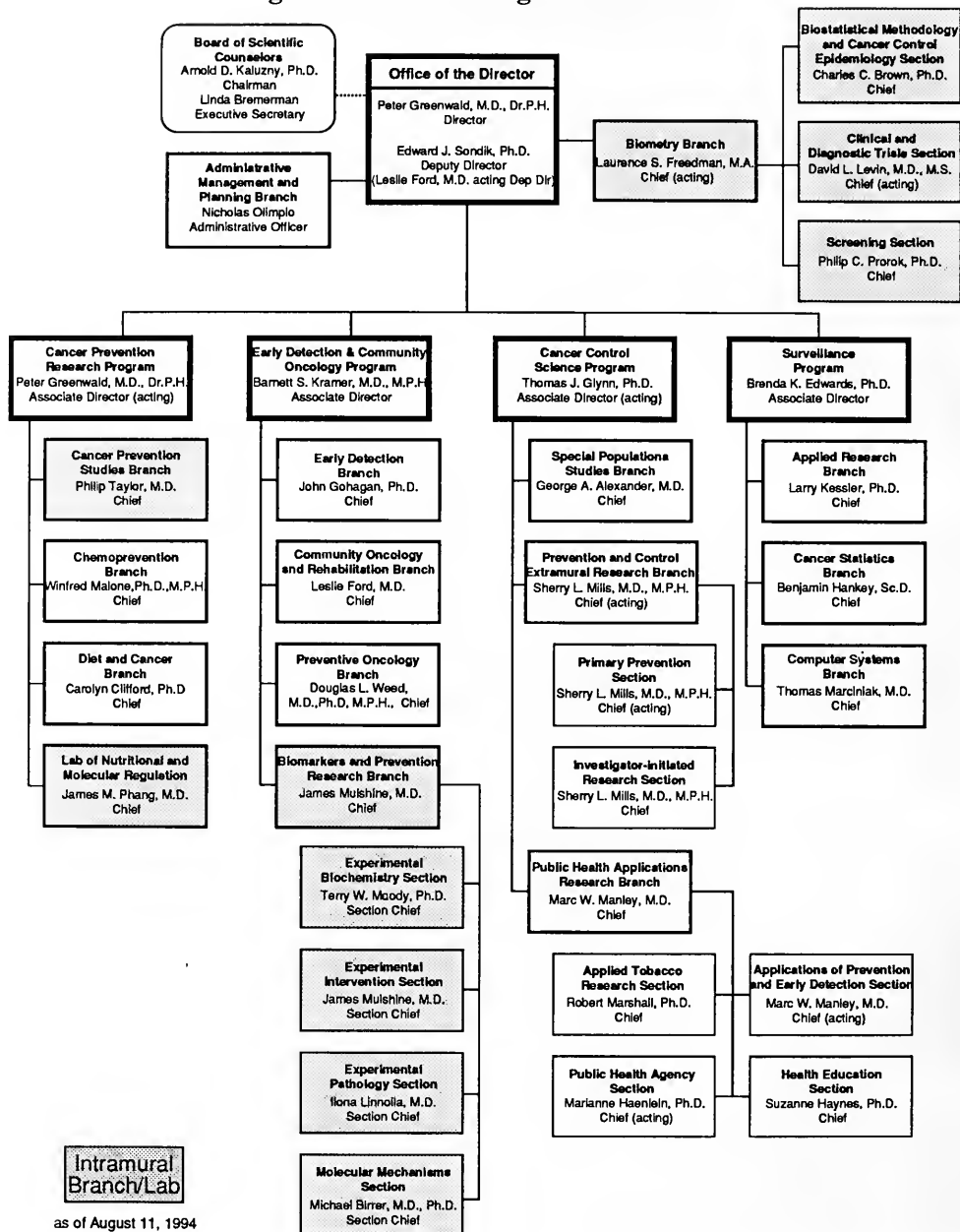
Figure 1 outlines the DCPC organization. The Division consists of four major programs, each led by an Associate Director. The Office of the Division Director provides overall coordination and direction as well as analytic program support. Each program is described briefly below.

The **Office of the Director** is responsible for the coordination and direction of the Division programs. It includes two branches: the Biometry Branch (one of the intramural components) and the Administrative Management and Planning Branch. The Biometry Branch conducts and supports intramural research using epidemiologic databases, intramural research in biostatistics methodology and aspects of cancer screening, and clinical trials research. The Administrative Management and Planning Branch assists in the management of the Division's budget and administrative matters.

The **Cancer Prevention Research Program (CPRP)** is charged with planning and supporting both intramural and extramural research in diet, nutrition and cancer, and chemoprevention. In addition, this organizational unit serves as the focal point for coordinating diet, nutrition, and cancer activities across the NCI divisions. This Program houses the Cancer Prevention Studies Branch (CPSB) and the Laboratory of Nutritional and Molecular Regulation (LNMR), the other two of the four intramural components of the Division. The Laboratory is located at the NCI-Frederick Cancer Research and Development Center (NCI-FCRDC) in Frederick, Maryland. The extramural units of the Program are the Chemoprevention Branch, responsible for agent identification, large-scale trials and investigator-initiated trials on chemoprevention regimens, and the Diet and Cancer Branch, which focuses investigator-initiated research on nutrition and diet, including the development of diet-based clinical trials.

The **Early Detection and Community Oncology Program (EDCOP)** supports the community-based clinical research programs, as well as early detection and rehabilitation research. The Program includes another of the Division's intramural components, the Biomarkers and Prevention Research Branch (BPRB). The Branch, located in an off-campus facility in Rockville, Maryland, focuses on the use of molecular biological techniques and clinical medicine to develop the biomarker tools to prevent and find cancer early. These programs are designed to improve the delivery and application of state-of-the-art cancer regimens.

Figure 1 — DCPC Organization Chart



as of August 11, 1994

The EDCOP extramural units include the Early Detection Branch, which supports research aimed at reducing cancer morbidity and mortality through appropriate early treatment; the Community Oncology and Rehabilitation Branch, which coordinates the Community Clinical Oncology Program (CCOP) and the minority-based Community Clinical Oncology Program—these CCOPs link community-based physicians with Cancer Centers and the Cooperative Groups to conduct clinical treatment research and cancer prevention and control research in community settings; and the Preventive Oncology Branch, which conducts the Division's Cancer Prevention Fellowship Program, providing an opportunity for physicians and scientists to train and gain field experience in cancer prevention and control by working with DCPC preceptors.

The **Cancer Control Science Program (CCSP)** supports research on ways to effectively transfer cancer control information to the public and to physicians, nurses, and other health professionals. This Program's efforts are directed toward study of a wide variety of cancer control intervention strategies to assess both their impact on populations and the use of proven cancer control methods. Programs that involve State, local and voluntary health groups, and populations that suffer disproportionately from cancer figure prominently in the Program's activities. The Program also directs a number of cancer control resource activities, including the National Black, Hispanic and Appalachian Leadership Initiatives on Cancer. The Program is organized into three extramural units: the Special Populations Studies Branch, the Public Health Applications Research Branch, and the Prevention and Control Extramural Research Branch. The Smoking and Tobacco Control Program, which provides liaison between NCI, NIH, and the Department of Health and Human Services, is another important component of the CCSP.

The **Surveillance Program** is responsible for tracking and evaluating trends in cancer and for research on quantitative methods and statistics designed to monitor progress in cancer control for the United States. An important part of the Surveillance Program is a network of population-based cancer reporting systems the Surveillance, Epidemiology, and End Results (SEER) Program. Related efforts gather and disseminate information on cancer, cancer risk factors, and other elements of cancer control through a variety of reports. The Program also conducts studies on the organization, delivery, and financing of cancer prevention and control services, as well as on the economics of cancer. The Surveillance Program includes three branches: the Cancer Statistics Branch, responsible for the SEER program; the Applied Research Branch, which conducts a variety of analytic and methodological studies including health services research and develops methods related to cancer surveillance and the evaluation of cancer control; and the Computer Systems Branch, which provides comprehensive computer systems analysis, design, operation, and programming support for the Division.

INTRAMURAL ACTIVITIES

The overall goal of the Intramural Program is to conduct research that provides the scientific basis for cancer prevention and control. In addition, the Program conducts methods research fundamental to the development of technical approaches underlying many large-scale projects in cancer prevention and control research. Within the DCPC, intramural research is conducted through the Biometry Branch (BB), the Cancer Prevention Studies Branch (CPSB), the Laboratory of Nutritional and Molecular Regulation (LNMR), and the Biomarkers and Prevention Research Branch (BPRB).

The **Biometry Branch** conducts research on epidemiologic methodology and investigates mathematical modeling of processes relevant to cancer prevention and control activities. The Biometry Branch also provides consultation on statistical methodology and study design within the Division and to other scientists both within and outside the NIH.

The **Cancer Prevention Studies Branch (CPSB)** conducts a variety of prevention research but has a focus on controlled intervention studies. Intervention studies serve the dual purposes of confirming hypotheses about cancer etiology and effecting cancer control, and act as a bridge between these two types of research efforts. The CPSB conducts intramural research in the areas of diet, nutrition and cancer, cancer chemoprevention, occupational cancer studies, and other cancer prevention strategies directed toward methods development and their application to reduce human cancer risk.

The **Laboratory of Nutritional and Molecular Regulation (LNMR)**, located at the NCI-Frederick Cancer Research and Development Center (NCI-FCRDC), conducts a broad range of studies including the use of drug-resistance as a model for cellular defense, nutrient-dependent modulation of signaling mechanisms in cell proliferation, and dietary perturbation of nutrient and carcinogen metabolism.

The **Biomarkers and Prevention Research Branch (BPRB)** uses molecular biological techniques and clinical medicine to develop the biomarker tools for use in cancer prevention and early detection trials. These programs are designed to improve the delivery and application of state-of-the-art cancer regimens.

All NCI intramural research is evaluated through a system of peer review. Committees of outstanding scientists representing the various disciplines involved in the intramural research program periodically review the direction and progress of the research program and staff. All of the intramural program is subject to the same critical review, including the review and approval of new research directions concepts prior to their implementation. The committees that review the intramural research address the breadth and depth of each project and its relation to the Division mission. Critiques also address the quality, progress, future directions, and an assessment of resources and staff development. Recommendations made at the peer reviews are monitored and the impact of their outcomes are assessed in subsequent site visits by the DCPC Board of Scientific Counselors.

In summary, the Division of Cancer Prevention and Control believes that a strong intramural research program complements the much larger extramural research portfolio. The program provides the facility to respond quickly to new directions, and to study and develop new paradigms for prevention and control research. The DCPC believes that an investment in intramural research is vital to the Nation's cancer prevention and control efforts. This Report outlines the program's progress and its promise.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Alpha-Tocopherol, Beta-Carotene Lung Cancer Prevention Study

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: D. Albanes Medical Officer CPSB, DCPC, NCI

Others:	P. R. Taylor	Branch Chief	CPSB, DCPC, NCI
	B. K. Edwards	Associate Director	SP, DCPC, NCI
	A. M. Hartman	Health Statistician	ARB, SP, DCPC, NCI
	S. B. Green	Lead Research Investigator	CDTS, BB, DCPC, NCI
	J. A. Tangrea	Deputy Branch Chief	CPSB, DCPC, NCI
	S. A. Glynn	Cancer Prevention Fellow	CPSB, DCPC, NCI
	L. S. Freedman	Acting Branch Chief	BB, DCPC, NCI
	S. G. Baker	Mathematical Statistician	SS, BB, DCPC, NCI
	C. C. Brown	Section Chief	BMCCES, BB, DCPC, NCI

COOPERATING UNITS (if any)

National Public Health Institute, Helsinki, Finland
Surveillance Program, DCPC
Biometry Branch, DCPC

LAB/BRANCH

Cancer Prevention Studies Branch, CPRP, DCPC

SECTION

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.5

PROFESSIONAL:

2.5

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human Subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Alpha-Tocopherol, Beta-Carotene Lung Cancer Prevention Study (ATBC Study) is investigating the efficacy of daily oral alpha-tocopherol (50 mg) and beta-carotene (20 mg) in a double-blind, randomized 2x2 factorial design trial aimed at preventing lung cancer among 50-69 year old male cigarette smokers. The project is based on experimental and epidemiological research which demonstrates a potential preventive role for these agents. Recruitment took place between 1985-88, and the trial intervention ended on schedule March 31, 1993 after an average followup of over 6 years. A postal survey screening for potential trial participants was sent to 291,000 men in southern Finland, and 76% responded. We invited the smokers willing to participate (43,000) to one of 13 study clinics, and over 29,000 were randomized into the study. Compliance to the one capsule daily regimen has remained very high (97% average), and the dropout rate averages less than 6% per year. Reduction of lung cancer incidence in the active agent groups is the primary study goal; differences in the occurrence of other cancers will also be evaluated. Several pilot studies in support of the trial have also been completed including a feasibility study, validation of study dietary questionnaires, and evaluation of skin yellowing and serum levels following beta-carotene administration.

This trial is being conducted collaboratively with the Surveillance Program and the Biometry Branch of the Division of Cancer Prevention and Control and with the National Public Health Institute of Finland.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Human Studies of Diet and Nutrition

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	P. R. Taylor	Branch Chief	CPSB, DCPC, NCI
	M. Forman	Nutritional Epidemiologist	CPSB, DCPC, NCI
	E. Lanza	Nutritionist	DCB, DCPC, NCI
	J. Dorgan	Senior Staff Fellow	CPSB, DCPC, NCI
Others:	A. Schatzkin	Medical Officer	CPSB, DCPC, NCI
	B. H. Patterson	Mathematical Statistician	BB, DCPC, NCI
	B. K. Edwards	Biostatistician	SP, DCPC, NCI
	W. Campbell	Research Study Coordinator	CPSB, DCPC, NCI
	M. Maher	Nurse Specialist	CPSB, DCPC, NCI
	C. C. Brown	Section Chief	BMCCES, BB, DCPC, NCI
	L. Yong	Staff Fellow	CPSB, DCPC, NCI
	B. I. Graubard	Mathematical Statistician	BB, DCPC, NCI

COOPERATING UNITS (if any)

U.S. Dept of Agriculture, Beltsville Human Nutrition Research Center
Surveillance Program, Biometry Branch, and Diet and Cancer Branch, DCPC
Armed Forces Institute of Pathology (M. Micozzi)

LAB/BRANCH

Cancer Prevention Studies Branch, CPRP, DCPC

SECTION

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

5.0

PROFESSIONAL:

4.25

OTHER:

0.75

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human Subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Among strategies to prevent cancer, clinical nutrition studies play a vital role in the transition from observational research to clinical trials. These studies 1) assess the metabolic effects of dietary changes in humans and 2) determine the safety, toxicity, pharmacokinetics, bioavailability, and mechanisms of action of macro- and micronutrients with chemoprevention potential, thereby providing information necessary to properly plan and conduct intervention studies using such agents or approaches. To further define these parameters in humans, a cooperative research effort between the Beltsville Human Nutrition Research Center in the U.S. Department of Agriculture and the CPSB, DCPC, was initiated in 1983. Since then, clinical nutrition research has been conducted in several areas but most prominently in relation to antioxidants and hormones. Antioxidant research has focused on dose, bioavailability, and safety of selenium, carotenoids, and vitamin C. Hormone research has focused on the potential modulating roles of dietary fat and alcohol. Altogether, over ten different projects have been conducted during this time. Future research will focus on important and promising areas of nutrition and cancer research, including antioxidants; fat, fiber, and energy; phytochemicals; and alcohol.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Use of Isotretinoin in Prevention of Basal Cell Carcinoma

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J. A. Tangrea Deputy Branch Chief CPSB, DCPC, NCI

Others: P. R. Taylor Branch Chief CPSB, DCPC, NCI
B. K. Edwards Associate Director SP, DCPC, NCI
A. M. Hartman Health Statistician ARB, DCPC, NCI
G. Peck Senior Investigator DB, DCT, NCI
C. C. Brown Section Chief BMCCES, BB, DCPC, NCI

COOPERATING UNITS (if any)

Walter Reed Army Med Ctr; Fitzsimmons Army Med Ctr; Brooke Army Med Ctr;
Eisenhower Army Med Ctr; Portsmouth Naval Med Ctr; Northwestern U; U of
Arkansas; Roswell Park Med Inst; Dermatology Br, NCI; Radiology Dept, Clinical Ctr;
Applied Research Branch, Surveillance Program, DCPC; Biometry Br, DCPC

LAB/BRANCH

Cancer Prevention Studies Branch, CPRP, DCPC

SECTION

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.2

PROFESSIONAL:

0.2

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human Subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This study is a 5-year, randomized, double-blind clinical trial designed to evaluate the effectiveness of low dosage levels of isotretinoin in reducing the incidence of basal cell carcinoma in a high-risk population, and to examine possible side effects associated with long-term administration of low doses of isotretinoin. A total of 981 subjects were entered over 36 months at 8 participating clinical centers located around the country. At each center, subjects were randomly allocated to intervention (10 mg/day) or control (placebo) groups. Active intervention concluded in June 1990.

The rationale for this study includes the following. Laboratory experiments have shown that retinoids administered to animals can prevent chemical carcinogenesis. In the experimental animals, retinoids were effective even if administered after exposure to the carcinogen, and therefore the prophylactic effect of the retinoids is believed to be in the postinitiation phase, i.e., during the promotion phase of carcinogenesis. Recent case reports have shown that isotretinoin can prevent the appearance of new basal cell carcinoma for 4 years in patients at higher risk of developing new tumors.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

NHANES I Epidemiologic Followup Survey: Chemoprevention/Nutrition Aspects

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: P. R. Taylor Branch Chief CPSPB, DCPC, NCI

Others:	D. Albanes	Medical Officer	CPSPB, DCPC, NCI
	A. Schatzkin	Medical Officer	CPSPB, DCPC, NCI
	J. Dorgan	Senior Staff Fellow	CPSPB, DCPC, NCI
	L. Yong	Staff Fellow	CPSPB, DCPC, NCI

COOPERATING UNITS (if any)

This research developed as a collaborative effort by NCHS and various institutes at NIH: Biometry Branch, DCPC, NCI; NIH; NIMH; NIAAA, NHLBI; NINDS; NIDDK; NIAID; National Center for Health Statistics

LAB/BRANCH

Cancer Prevention Studies Branch, CPRP, DCPC

SECTION

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.5

PROFESSIONAL:

0.5

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human Subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of the NHANES (National Health and Nutrition Examination Survey) epidemiologic followup survey was to conduct a longitudinal study of 14,407 adults originally surveyed in 1971-75 and to investigate subsequent health and mortality outcomes. Respondents were traced and re-examined. Information was obtained from hospital records, the National Death Index, and death certificates. Several cycles have now been performed. The initial NHANES followup survey was completed in 1984. A continued followup of the elderly (75 years of age or older) in this cohort was conducted in 1985-86, while the entire cohort was again followed in 1986-87. Further followup in 1992 is near completion.

The purpose of this intramural project is to examine the relation of chemopreventive, nutritional, and constitutional factors to cancer in the very large, representative population which NHANES offers. It provides an opportunity to examine these factors and potentially confounding or modifying factors in a prospective fashion, and to examine the effectiveness of dietary agents which are currently of great interest for cancer prevention. The relation of baseline vitamin use, biochemical or nutritional measures, and subsequent health status will be examined.

This study is being conducted by several of the National Institutes of Health and the National Center for Health Statistics.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Research in Cancer Screening and Statistical Methodology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PIs:	P. C. Prorok	Section Chief	SS, BB, DCPC, NCI
	R. J. Connor	Mathematical Statistician	SS, BB, DCPC, NCI
	S. G. Baker	Mathematical Statistician	SS, BB, DCPC, NCI
	R. Fagerstrom	Mathematical Statistician	SS, BB, DCPC, NCI
Others:	K. Kafadar	Guest Researcher	SS, BB, DCPC, NCI
	J. L. Xu	Visiting Fellow	SS, BB, DCPC, NCI
	D. L. Weed	Branch Chief	POB,EDCOP,DCPC,NCI
	D. Friedman	Epidemiology and Biostatistics Fellow	REB, DCE, NCI

COOPERATING UNITS (if any)

Prevention Oncology Branch, DCPC, NCI; Radiation Epidemiology Branch, DCE, NCI; Department of Public Health and Social Medicine, Erasmus University, Rotterdam, the Netherlands (G. van Oortmarssen, R. Boer)

LAB/BRANCH

Biometry Branch, OD, DCPC

SECTION

Screening Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

3.0

PROFESSIONAL:

2.6

OTHER:

0.4

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human Subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

Analysis of data originally obtained from Human Subjects/Human Tissue

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The focus of this project is development and refinement of statistical procedures for the design and analysis of cancer screening and related studies. Problems under investigation include derivation and comparison of data analysis methods, assessment of case-control studies for screening evaluation, development of models of cancer screening, approaches to the evaluation of diagnostic tests, and estimation and adjustment of lead time in cancer survival data. To assess the case-control design for screening evaluation, the MISCAN microsimulation model is being used to provide population data with and without screening. Case-control studies are then done in the screened populations, and the results are compared with the true effect to assess bias in the case-control approach. Criteria were developed for comparability of the restricted case subgroups used in the Limited Analysis of a cancer screening trial. A method for estimating the effect of starting periodic screening at different ages was developed that uses data from a screened population to estimate the probability of diagnosis in an unscreened group. A new method was derived for estimating the true and false positive rates of multiple diagnostic tests accounting for unrecorded variables that may be related to the decision to verify disease. Methods have been developed for estimating the benefit of screening unaffected by lead time bias and the average lead time by examining the differences in case survival measured both from time of entry and time of diagnosis between screened and control groups. Variance estimators for these estimators were derived. Approaches for estimating the post lead time survival of screen detected cancer cases were developed. Approaches were defined for data monitoring of cancer screening trials, including sequential methods for nonproportional hazards techniques and a Bayesian method.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies in Cancer Screening

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PIs:	P. C. Prorok	Section Chief	SS, BB, DCPC, NCI
	R. J. Connor	Mathematical Statistician	SS, BB, DCPC, NCI
	S. G. Baker	Mathematical Statistician	SS, BB, DCPC, NCI
	R. Fagerstrom	Mathematical Statistician	SS, BB, DCPC, NCI
Others:	J. K. Gohagan	Branch Chief	EDB, DCPC, NCI
	L. Kessler	Branch Chief	ARB, DCPC, NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Biometry Branch, OD, DCPC

SECTION

Screening Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.7

PROFESSIONAL:

1.3

OTHER:

0.4

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human Subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Data from several cancer screening studies are being collected and analyzed to gain a better understanding of the impact and consequences of such screening in various population settings, and to develop new techniques for data analysis. Section staff are involved in design, monitoring, and data analysis aspects of these studies.

Screening Section investigators collaborated with the Early Detection Branch and the Research Contracts Branch in developing the major components of the PLCO Trial. This is a major trial of cancer screening in males and females for four cancers that comprise more than 50% of the incidence and mortality of cancer: lung, prostate, colorectal, and ovarian cancers. The trial design calls for a total sample size of 74,000 males and 74,000 females between the ages of 60 and 74 who are to be divided at random into a screened group and a control group. The screening techniques to be used are annual digital rectal examination and prostate specific antigen for prostate cancer, annual chest film for lung cancer, three-yearly flexible sigmoidoscopy for colorectal cancer, and annual ovarian physical examination plus CA-125 marker and transvaginal ultrasound for ovarian cancer. Enrollment of trial participants and initial screening of those randomized to the intervention arm for the Pilot Phase of the trial began in November, 1993. Preliminary data on recruitment, compliance, contamination, and test results were analyzed.

The database from the HIP breast cancer screening trial was used to address several scientific and modeling issues. Issues under investigation included the magnitude and duration of benefit, age-specific effectiveness, and application to model development and validation. The colorectal cancer screening trial at the University of Minnesota is designed to evaluate testing for occult blood in the stool as an early detection maneuver for colorectal cancer. Staff are involved in monitoring this trial which reported a 32% reduction in colorectal cancer mortality in the screened arm.

Section staff are involved in the International Workgroup on Information Systems in Breast Cancer Detection. The goal is development of a database containing key data elements from countries doing breast screening that could be used jointly or individually by the countries for evaluation of breast cancer detection. An initial database questionnaire has been developed, terminology has been refined, and initial collection of data was begun in several countries.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Design and Analysis of Pharmacokinetic Studies of Selenium

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: B. H. Patterson Mathematical Statistician BMCCES, BB, DCPC, NCI
Others: L. A. Zech Senior Scientist LMMB, DCBD, NCI

COOPERATING UNITS (if any)

Laboratory of Mathematical Biology, DCBD
Cancer Prevention Studies Branch, DCPC

LAB/BRANCH

Biometry Branch, OD, DCPC

SECTION

Biostatistical Methodology and Cancer Control Epidemiology Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.5

PROFESSIONAL:

0.4

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human Subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Selenium is a possible cancer preventive agent. A study is in progress to provide information on the pharmacokinetics of selenium in its prototype forms: sodium selenite and selenomethionine. This information is necessary for the determination of time and manner of administration.

Integrated kinetic models are being used to interpret the study data. Various body pools have been hypothesized and rates of exchange between them estimated, as well as residence times. The models indicate important kinetic differences between selenite and selenomethionine. Alternative models were used to investigate one of the most important differences, the recirculation of the organic, but not the inorganic. The models have been modified and combined into a single model to better simulate dietary intake of selenium.

A workshop, organized jointly with the Chemoprevention Investigational Drug Unit, was held to review the current state of knowledge on the efficacy and toxicity of selenium compounds in preventing cancer in animals and humans. Leading selenium researchers gave presentations providing an overview of the current state of selenium research. Se was regarded in general as a safe substance. Toxicities seen at very high levels of intake were reversible with the discontinuance of the high dose. Animal data were regarded as of relatively minor interest as good human data are available.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Nutrition Intervention Studies of Esophageal Cancer in Linxian, China

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PIs:	P. R. Taylor	Branch Chief	CPSB, DCPC, NCI
	W. Blot	Branch Chief	BB, DCE, NCI
Others:	J. A. Tangrea	Deputy Branch Chief	CPSB, DCPC, NCI
	S. Dawsey	Senior Staff Fellow	CPSB, DCPC, NCI
	S. Mark	Staff Fellow	BB, DCE, NCI
	M. Gail	Section Chief	BB, DCE, NCI

LAB/BRANCH

Cancer Prevention Studies Branch, CPRP, DCPC

SECTION

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.0

PROFESSIONAL:

2.0

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human Subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to conduct two intervention trials using multiple vitamin-mineral supplements to evaluate the relationship between such supplements and esophageal cancer incidence and mortality. One trial is being conducted in patients diagnosed with esophageal dysplasia (n=3,400) and the other in the general population in a high-risk region (n=30,000). The effect of these supplements on regression/progression of esophageal dysplasia and total cancer incidence, total cancer mortality, and total mortality will be evaluated. These two studies are being conducted in Linxian (Henan Province) in the People's Republic of China (PRC). Linxian, a rural country with population 800,000 was selected because it has the highest rate of esophageal cancer in the world (greater than 100/100,000) and because there is suspicion that the population's chronic deficiencies of multiple nutrients may be etiologically involved. Active intervention concluded in May 1991, but post-intervention followup continues.

This study is being conducted jointly by the Biostatistics Branch of the Division of Cancer Etiology and the Cancer Prevention Studies Branch of the Division of Cancer Prevention and Control at the NCI in collaboration with the Cancer Institute of the Chinese Academy of Medical Sciences.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Statistical Methodology Research

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PIs:	S. B. Green	Lead Research Investigator	CDTS, BB, DCPC, NCI
	B. I. Graubard	Mathematical Statistician	CDTS, BB, DCPC, NCI
Others:	D. K. Corle	Computer Systems Analyst	CDTS, BB, DCPC, NCI

COOPERATING UNITS (if any)

Applied Research Branch, SP, DCPC, NCI, National Center for Health Statistics,
Information Management Services, Inc., University of Maryland

LAB/BRANCH

Biometry Branch, OD, DCPC

SECTION

Clinical and Diagnostic Trials Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.5

PROFESSIONAL:

1.5

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human Subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to conduct independent research in statistical methods and computer techniques with particular emphasis on those appropriate for analyzing data from clinical, diagnostic, and prevention trials and epidemiologic studies of cancer. Many of the problems studied under this project arise from the consultative activities of the Section. Among the current projects are:

Analyzing Disease Progression with Status Measurements at Fixed Time Points: Use of Permutation Tests. In intervention trials in which patient status is measured regularly as an indication of disease progression, we often want an overall measure of impairment over time. Such measures are particularly of interest in the absence of a specific irreversible event whose incidence or time-to-occurrence can be compared between groups.

Comparison of Methods for Identifying Prognostic Factors and Predicting Survival for Patients with Colorectal Cancer. In collaboration with a working group of the American Joint Committee on Cancer, work has continued on fitting Cox proportional hazards models to a dataset on patients with colorectal cancer. The goal is to identify important prognostic factors and then apply these to predict survival probabilities at various points of time.

Methods for Analyzing Complex Survey Data. Data from household surveys are from clustered samples of persons who are often selected at differential rates. These aspects of the sampling result in nonindependence and unequal weighting of the observations that should be considered during the analysis stage. Survey data are used extensively in cohort studies through long term followup of the sample, case-control studies by providing population controls, and cross-sectional studies. Other projects involve collaboration with the Division of Cancer Treatment and the NCHS.

Analysis of Diet Survey Data: Typical Consumption and Effects of Covariates. In collaboration with the University of Maryland, a parametric statistical model was constructed to model count data provided by 24-hour recall questionnaires. The model permits one to use regression models to relate abstention and average consumption to covariates such as income, race, day of week, or season. An important feature is that the model separates within and between person variation of count data. As a result, one can estimate the distribution of typical individual consumption, either for the entire population or for selected subpopulations.

Interactive Statistical Programs. The Section continues to maintain and improve a group of interactive computer programs for efficient analysis of medical data, particularly those dealing with risk factors and prognostic factors using sophisticated multiple regression techniques and survival analysis.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Consultation on Clinical Trials and Other Studies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PIs:	S. B. Green	Lead Research Investigator	CDTS, BB, DCPC, NCI
	D. K. Corle	Computer Systems Analyst	CDTS, BB, DCPC, NCI
	B. I. Graubard	Mathematical Statistician	CDTS, BB, DCPC, NCI

COOPERATING UNITS (if any)

Early Detection and Community Oncology Program, Cancer Control Science Program, & Cancer Prevention Research Program, DCPC; Division of Cancer Treatment & Division of Cancer Etiology, NCI; Information Management Services, Inc.

LAB/BRANCH

Biometry Branch, OD, DCPC

SECTION

Clinical and Diagnostic Trials Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human Subjects ☐ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to provide independent consultation on statistical and epidemiological methodology in the design, interpretation, and evaluation of clinical trials of diagnosis, treatment, and prevention of cancer, and other studies requiring this kind of expertise. For some studies the Section provides full statistical support, including development of detailed study plans; assistance in the design of appropriate study forms; supervision of randomization (for trials); collection, processing, and editing of data; performance of interim analyses during the progress of the study; preparation of progress reports; final analysis of study data; and collaboration in the preparation of scientific papers. Among the current projects are:

HIV Prevention Trials. Consultation on intervention trial design is being provided to the NIAID via membership on several working groups dealing with various aspects of the prevention of HIV infection and AIDS.

Analysis of USDA Feeding Studies. We are collaborating with the Cancer Prevention Studies Branch on the analysis of a series of USDA feeding studies of women 20-40 years of age. Research is underway to study the complex relationships between blood lipids, hormone levels, and plasma carotenoid levels by phase of the menstrual cycle.

Study of the Adoption and Use of the Primary Care Nutrition Guide. The Section has provided statistical consultation to the Public Health Applications Research Branch on the design, planning, and implementation of a physician practice study evaluating the NCI Primary Care Nutrition Guide and a training course (based on the Guide) among internal medicine and family medicine practices.

Working Well Project. In collaboration with the Prevention and Control Extramural Research Branch, the Coordinating Center, and the Evaluation Working Group, study endpoints (smoking and dietary) were defined, with particular emphasis on who should be included in the analysis.

Other Projects. A number of other projects both within the DCPC and with many outside researchers such as the University of Texas, the University of Arkansas, the National Institute of Child Health and Human Development, the National Institute on Alcohol Abuse and Alcoholism, Harvard University, the Division of Cancer Treatment, the National Center for Health Statistics, and Battelle Pacific Northwest Laboratory are also being pursued.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Research in Biostatistical Methodology and Mathematical Modeling

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	C. C. Brown	Section Chief	BMCCES, BB, DCPC, NCI
Others:	V. Kipnis	Visiting Scientist	BMCCES, BB, DCPC, NCI
	L. S. Freedman	Acting Branch Chief	BB, DCPC, NCI
	A. Schatzkin	Medical Officer	CPSB, DCPC, NCI
	S. Wacholder	Mathematical Statistician	BB, DCE, NCI
	A. M. Hartman	Health Statistician	ARB, DCPC, NCI

COOPERATING UNITS (if any)

Cancer Prevention Studies Branch, CPRP, DCPC, NCI
Biostatistics Branch, EBP, DCE, NCI
Applied Research Branch, SP, DCPC, NCI

LAB/BRANCH

Biometry Branch, OD, DCPC

SECTION

Biostatistical Methodology and Cancer Control Epidemiology Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.9

PROFESSIONAL:

0.8

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human Subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Different statistical methods to adjust the effect of macronutrient intake for total energy intake are currently being used to analyze epidemiologic studies of diet and disease. This research examines the statistical properties of these methods. Published during the past year were:

- 1) a paper which interprets the regression coefficients of three alternative regression models showing that four different effects of interest (related to either adding calories to the diet or substituting sources of calories in the diet) are estimable by each model and derives the standard errors of these estimates; and
- 2) a paper which examines the behavior of these methods when the study subjects are categorized into a small number of groups according to their nutrient intake. When the true macronutrient intakes and their inter-relationships are known without error, this investigation shows that Willett's "residual" method is more powerful than the "standard" method (both measuring the effect of calorie substitution) and very similar to the "density" method.

Submitted for publication is a paper which examines the ability of currently used energy adjustment regression methods to disentangle the effects of total energy from its component macronutrient-specific parts. We determined that, based solely on results from a diet-cancer epidemiologic study, concluding the existence of a specific macronutrient effect as opposed to a generic energy effect is not possible without making additional assumptions.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cancer Control Objectives and Cancer Mortality Projections

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	D. L. Levin	Senior Research Investigator	BB, DCPC, NCI
Others:	L. G. Kessler	Branch Chief	ARB, SP, DCPC, NCI
	A. Potosky	Operations Research Analyst	ARB, SP, DCPC, NCI
	M. Brown	Economist	ARB, SP, DCPC, NCI
	E. Feuer	Mathematical Statistician	ARB, SP, DCPC, NCI

COOPERATING UNITS (if any)

Applied Research Branch, Surveillance Program, DCPC
Information Management Services, Inc.

LAB/BRANCH

Biometry Branch, OD, DCPC

SECTION

Office of the Chief

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.0

PROFESSIONAL:

1.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human Subjects ☐ (b) Human tissues ☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Projecting cancer incidence and mortality rates, and relating those projections to the attainment of national cancer control objectives, are the goals of this intramural research project. The project includes development and continued refinement of a computer model which projects cancer incidence and mortality, meshing together data from a variety of sources, and adapting quantitative cancer control objectives to fit the modeling framework.

The NCI staff has developed and written a large interactive mainframe computer program used to project cancer figures for a forty year period. The model incorporates different models for survival from cancer, includes data for a number of cancer sites, the ability to examine temporal trends in underlying cancer incidence and mortality from other causes, adjustment of rates to different populations, and production of annual projections of cancer incidence and mortality. The crux of the model is the flexibility to analyze the effect of cancer prevention, screening, and treatment activities (in any combination) on cancer mortality. A similar program which runs on an IBM desktop computer is being developed which incorporates additional features related to cost of cancer.

Work in the current year has involved revision and updating of the desktop computer version with a new interface and extensive use of help screens.

PERIOD COVERED October 1, 1993 to September 30, 1994		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Continued Followup of the Breast Cancer Detection and Demonstration Project		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PIs:	A. Schatzkin C. Schairer	Medical Officer Health Statistician CPSB, DCPC, NCI EEB, DCE, NCI
Others:	R. N. Hoover L. A. Brinton L. Yang	Branch Chief Section Chief Staff Fellow EEB, DCE, NCI EEB, DCE, NCI CPSB, DCPC, NCI
COOPERATING UNITS (if any) Environmental Epidemiology Branch, DCE Biometry Branch, DCPC		
LAB/BRANCH Cancer Prevention Studies Branch, CPRP, DCPC		
SECTION		
INSTITUTE AND LOCATION National Cancer Institute, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 1.5	PROFESSIONAL: 1.0	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human Subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) MInors <input checked="" type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The Breast Cancer Detection and Demonstration Project (BCDDP) screening program began in 1973 in 29 centers in 27 widely dispersed geographic areas of the United States. Initial screening was complete on over 280,000 women over a 2-year period. From the original 280,000 participants in the screening phase of the BCDDP, approximately 64,000 were selected for 4 years of long-term followup (LTF) beginning in 1978, to assess the biology and natural history of breast disease, and to test hypotheses relating to detection, etiology, and survival. Those selected for LTF included all breast cancer cases found during the screening phase, all benign breast cancer cases, all those recommended for biopsy, and a sample of "normals." The LTF database will facilitate the exploration of important questions regarding the etiology and natural history of breast cancer. The size of the subcohorts and breadth of data available on them make this population unique. The large number of cases of both breast cancer and benign breast disease with histologic information available should allow particularly useful analyses of several risk factors in relation to these conditions.</p> <p>The first 5 years of LTF was completed in all centers in September 1986. Further continued followup occurred in 1988-89 and again in 1993-94.</p> <p>This project is being conducted jointly by the Cancer Prevention Studies Branch and the Biometry Branch of the Division of Cancer Prevention and Control and the Environmental Epidemiology Branch of the Division of Cancer Etiology.</p>		

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Nutritional Factors and Cancer in the Framingham Heart Study

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PIs:	A. Schatzkin	Medical Officer	CPSB, DCPC, NCI
	J. Dorgan	Senior Staff Fellow	CPSB, DCPC, NCI

COOPERATING UNITS (if any)

Boston University and the National Heart, Lung and Blood Institute (NHLBI)

LAB/BRANCH

Cancer Prevention Studies Branch, CPRP, DCPC

SECTION

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- | | | |
|--|--|--------------------------------------|
| <input checked="" type="checkbox"/> (a) Human Subjects | <input type="checkbox"/> (b) Human tissues | <input type="checkbox"/> (c) Neither |
| <input type="checkbox"/> (a1) Minors | | |
| <input checked="" type="checkbox"/> (a2) Interviews | | |

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In recent years considerable interest has been focused on the possible relation between moderate consumption of alcoholic beverages and breast cancer in women. Five epidemiologic cohort studies and the majority of case-control studies have demonstrated a positive association between moderate alcohol consumption and breast cancer, with relative risks ranging from 1.5 to 2.0. Given the frequency of alcohol consumption among women in this country, even a risk elevation of 50-100% would translate into considerable breast cancer morbidity and mortality that would be attributable to drinking. Further epidemiologic investigation of this question is of high priority.

In this regard, in 1985, the Division of Cancer Prevention and Control funded a contract for the procurement of a cancer file based on the original cohort in the Framingham Heart Study. This ongoing prospective cohort study was initially set up to examine risk factors for coronary heart disease, stroke, and other cardiovascular endpoints. Data, including detailed information on alcohol consumption, have been collected for over 40 years. The cancer file was used to examine a number of hypotheses relating nutritional factors to cancer, including alcohol use, body fat distribution, physical activity, and serum cholesterol. A contract to update this cancer file with diagnoses subsequent to completion of the initial file was funded this year.

A similar study (Z01 CN 00147-06 CPSB) is being conducted on children of the original cohort.

This study is being conducted collaboratively with investigators from Boston University and the NHLBI.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Nutritional Factors and Cancer in the Framingham Offspring Study

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PIs:	A. Schatzkin	Medical Officer	CPSB, DCPC, NCI
	J. Dorgan	Senior Staff Fellow	CPSB, DCPC, NCI

COOPERATING UNITS (if any)

Boston University and the National Heart, Lung and Blood Institute (NHLBI)

LAB/BRANCH

Cancer Prevention Studies Branch, CPRP, DCPC

SECTION

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.50

PROFESSIONAL:

0.25

OTHER:

0.25

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human Subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Framingham Offspring Study was undertaken in order to explore the relation between alcohol and breast cancer. This cohort study consists of 5,135 children (2,646 female, 2,489 male) of the members of the original Framingham Heart Study Cohort. The baseline examination period was 1972-77 (Cycle 1). Subsequent followup periods were 1979-82 (Cycle 2), 1984-5 (Cycle 3), and 1987-9 (Cycle 4). Alcohol consumption, both frequency and amount by type of beverage, has been ascertained at each cycle. Information on socioeconomic status, and reproductive and family history has been routinely collected. These additional data are important in controlling for variables that might confound an observed association between alcohol and breast cancer.

Ascertainment of cancers currently is partially complete through cycle 3. A contract to update the cancer file with diagnoses subsequent to completion of the initial file was funded this year.

A similar study (Z01 CN 00146-06 CPSB) is being conducted on the original cohort.

This study is being conducted collaboratively with investigators from Boston University and the NHLBI.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Finland Studies of Nutrition and Cancer

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	D. Albanes	Medical Officer	CPSB, DCPC, NCI
Others:	P. R. Taylor	Branch Chief	CPSB, DCPC, NCI
	B. K. Edwards	Associate Director	SP, DCPC, NCI
	A. M. Hartman	Health Statistician	ARB, DCPC, NCI

COOPERATING UNITS (if any)

National Public Health Institute, Finland
Social Insurance Institute, Finland
Applied Research Branch, Surveillance Program, DCPC

LAB/BRANCH

Cancer Prevention Studies Branch, CPRP, DCPC

SECTION

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.3

PROFESSIONAL:

0.3

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human Subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The important relationship of diet and nutrition in the development of cancer has become well known through various research efforts. Laboratory studies have shown cancer inhibitory function for various natural and synthetic nutrients in various models, which have been corroborated by human epidemiologic studies of nutrient intake, tissue levels, and cancer incidence. The objectives of these etiologic studies are to 1) assess the role of fats; selenium; and vitamins A, E, and C in breast cancer development; and 2) evaluate the relation of intake of various nutrients to subsequent cancer, particularly breast, colon, and lung. The project includes two studies. The first is a breast cancer case-control study of fats; total calories; selenium; and vitamins A, E, and C. The role of various anthropometric measurements, genetic markers for breast cancer, and reproductive factors are being explored. The second project is a comparison of nutrient intakes in cases and reference subjects identified from an existing large cohort with prediagnostic baseline dietary histories. Associations between various dietary components and several cancers will be assessed.

These studies are being conducted collaboratively with the Surveillance Program of the Division of Cancer Prevention and Control and the National Public Health Institute and Social Insurance Institute of Finland.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Yunnan Tin Miners Lung Cancer Studies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	P. R. Taylor	Branch Chief	CPSB, DCPC, NCI
Others:	A. Schatzkin	Medical Officer	CPSB, DCPC, NCI
	M. Forman	Nutritional Epidemiologist	CPSB, DCPC, NCI
	Y. L. Qiao	Visiting Associate	CPSB, DCPC, NCI
	B. I. Graubard	Mathematical Statistician	BB, DCPC, NCI
	M. M. Maher	Nurse Specialist	CPSB, DCPC, NCI

COOPERATING UNITS (if any)

Yunnan Tin Corporation
Cancer Institute, Chinese Academy of Medical Sciences
Division of Cancer Etiology, NCI
Biometry Branch, DCPC, NCI

LAB/BRANCH

Cancer Prevention Studies Branch, CPRP, DCPC

SECTION

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human Subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

As part of our general collaborative studies in China and the feasibility study for a lung cancer intervention study among Yunnan tin miners, two lung cancer case-control studies have been conducted among the tin miners. The first, a prevalence case-control study, interviewed 107 living cases diagnosed between 1967-84 and an equal number of matched controls. A second study includes 183 lung cancer cases incident in 1985 and 1986 among miners and an equal number of matched controls. Data concerning smoking, occupational exposures including radon and arsenic exposure, diet and other exposures were collected by personal interview. Analyses of risk by radon, tobacco, and arsenic in the prevalence study have been completed while analyses of the incident case-control study are ongoing.

These studies are being conducted collaboratively with scientists from the Cancer Institute of the Chinese Academy of Medical Sciences, the Labor Protection Institute of the Yunnan Tin Corporation, and the Division of Cancer Etiology at NCI.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Esophageal Cancer Genetics Studies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	P. R. Taylor N. Dracopoli	Branch Chief Program Chief	CPSB, DCPC, NCI PTD, GBR, NCHGR
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Others:	S. Dawsey N. Hu	Senior Staff Fellow Cancer Prevention Fellow	CPSB, DCPC, NCI CPSB, DCPC, NCI
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COOPERATING UNITS (if any)

Chinese Academy of Medical Sciences
National Center for Human Genome Research
Fox Chase Cancer Center

LAB/BRANCH

Cancer Prevention Studies Branch, CPRP, DCPC

SECTION

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.5

PROFESSIONAL:

0.5

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human Subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The overall goal of this project is to develop an understanding of the genetic as well as environmental influences that are involved in the etiology of human esophageal cancer. These studies are being conducted in North Central China where rates of esophageal cancer are highest in the world, and where extraordinary familial aggregation of the disease exists.

A series of studies to evaluate the role of genetics in the etiology of esophageal cancer have been conducted, including studies of family history of esophageal cancer, familial aggregation, and segregation analyses of family pedigrees from high risk families. Future studies will search for esophageal cancer susceptibility genes through genomic searches of tumor/nontumor tissue and prospective followup of high risk families for linkage analysis.

This study is being conducted collaboratively with scientists at the Chinese Academy of Medical Sciences, the National Center for Human Genome Research, and the Fox Chase Cancer Center.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

A Dietary Intervention Study of the Recurrence of Large Bowel Adenomatous Polyps

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	A. Schatzkin	Medical Officer	CPSB, DCPC, NCI
	E. Lanza	Nutritionist	CPSB, DCPC, NCI
Others:	L. S. Freedman	Acting Branch Chief	BB, DCPC, NCI
	C. Clifford	Health Scientist Administrator	DCB, DCPC, NCI
	J. Tangrea	Deputy Branch Chief	CPSB, DCPC, NCI
	R. Ballard-Barbash	Medical Officer	ARB, DCPC, NCI

COOPERATING UNITS (if any)

Biometry Branch and Diet & Cancer Branch, DCPC; U of Pittsburg (Pittsburg, PA); Kaiser Found Res Inst (Oakland, CA); Memorial Sloan Kettering Cancer Ctr (New York, NY); U of Illinois (Chicago, IL); Kaiser Found (Portland, OR); U of New York (Buffalo, NY); Walter Reed Army Med Ctr (Washington, DC); U of Utah (Salt Lake City, UT); and Edward Hines Jr. VA Hospital (Chicago, IL)

LAB/BRANCH

Cancer Prevention Studies Branch, CPRP, DCPC

SECTION

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

4.0

PROFESSIONAL:

4.0

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human Subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Large bowel adenomatous polyps present a unique opportunity to conduct an intervention trial because of the high prevalence rate in the general population, the high polyp recurrence rate in those who have undergone polypectomy, and the link between polyps and cancer. It is generally accepted that large bowel adenomas are a requisite precursor lesion for most large bowel cancers. Given the strong evidence for the polyp-cancer sequence, an intervention that reduces the recurrence of large bowel polyps would have a strong likelihood of reducing the incidence of large bowel cancer.

The major objective of this study is to determine whether a low fat, high fiber, high fruit and vegetable dietary pattern will decrease the recurrence rate of large bowel adenomatous polyps. This is a multi-center randomized controlled trial involving 2,000 men and women. Study participants are being randomized into either the experimental diet group or a control group (usual diet). Recruitment will take up to two years, and the followup time from randomization is four years.

The study has three secondary objectives: 1) to investigate the relation between the dietary intervention and several putative intermediate endpoints in large bowel carcinogenesis, particularly markers of colonic epithelial cell proliferation; 2) to evaluate whether these intermediate endpoints correlate with subsequent neoplasia (adenoma formation); and 3) to determine the extent to which changes in the intermediate endpoints account for the observed effect.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Evaluation of the Effects of a Fat-Modified Diet on Hormones During Adolescence

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J. Dorgan	Senior Staff Fellow	CPSB, DCPC, NCI
Others:	A. Schatzkin	Medical Officer	CPSB, DCPC, NCI
	P. R. Taylor	Branch Chief	CPSB, DCPC, NCI
	B. H. Patterson	Mathematical Statistician	BB, DCPC, NCI

COOPERATING UNITS (if any)

National Heart, Lung, & Blood Institute; Children's Hospital (New Orleans, LA); Johns Hopkins U (Baltimore, MD); Kaiser Center for Health Res (Portland, OR); Maryland Med Res Inst (Baltimore, MD); Med College of New Jersey (Newark, NJ); Northwestern U (Chicago, IL); U of Pittsburgh (Pittsburgh, PA); U of Iowa (Iowa City, IA)

LAB/BRANCH

Cancer Prevention Studies Branch, CPRP, DCPC

SECTION

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.75

PROFESSIONAL:

0.75

OTHER:

0.25

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human Subjects ☐ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This study is ancillary to the Diet Intervention Study in Children (DISC), sponsored by the Division of Epidemiology and Clinical Applications, National Heart, Lung, and Blood Institute (NHLBI). DISC is a multicenter, randomized clinical trial designed to evaluate the feasibility, safety and efficacy of a fat modified diet during adolescence to lower LDL-cholesterol. The NCI sponsored ancillary study will evaluate the effect of this fat modified diet on sex hormones during adolescence. The effect of the diet on total concentrations of hormones and bioavailable fractions of hormones will be determined. The NCI sponsored ancillary study will also identify characteristics of adolescents, including age, Tanner stage, anthropometric measures, physical activity and dietary intake that affect sex hormone levels and bioavailability of sex hormones.

Dietary goals for the intervention group are to limit fat intake to 28% of calories and increase the ratio of polyunsaturated to saturated fats to approximately 1. Cholesterol intake will be restricted to 75mg/1000 calories. Children in the control group follow their usual diets.

This study is being conducted collaboratively with scientists from the National Heart, Lung, and Blood Institute in Bethesda, MD; Children's Hospital in New Orleans, LA; the Johns Hopkins University in Baltimore, MD; the Kaiser Center for Health Research in Portland, OR; the Maryland Medical Research Institute in Baltimore, MD; the Medical College of New Jersey in Newark, NJ; Northwestern University in Chicago, IL; the University of Pittsburgh in Pittsburgh, PA; and the University of Iowa in Iowa City, IA.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Fels Early Nutrition and Growth Study

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: D. Albanes Medical Officer CPSB, DCPC, NCI

COOPERATING UNITS (if any)

Wright State School of Medicine

LAB/BRANCH

Cancer Prevention Studies Branch, CPRP, DCPC

SECTION

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.1

PROFESSIONAL:

0.1

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human Subjects ☐ (b) Human tissues ☐ (c) Neither☐ (a1) Minors☒ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

This project is designed to investigate the relation of childhood nutrition to breast cancer risk factors, including age at menarche, adult height, weight, and fatness. Secondary purposes include tracking the development of overweight and obesity from birth through young adulthood, identification of possible "sensitive" or high-risk periods (with respect to obesity) in childhood and, more important, to identify the contribution of diet to the development of childhood and adult obesity.

Detailed anthropometric data (height, weight, skinfold thickness, etc.) and demographic characteristics available from a computer data base from the Fels Study and the Division of Human Biology of the Wright State School of Medicine included up to 18 annual dietary and anthropometric assessments for 106 girls. Calorie, macro- and micronutrient data were linked to the existing anthropometry computer file, including later adult height and weight. Nutrient composition has been calculated using the latest version of the USDA Handbook series. Nutrients include the following: total energy (kilocalories); total fat, protein, and carbohydrate; saturated, polyunsaturated, and monounsaturated fat; cholesterol; dietary fiber; and vitamins and minerals (from food and supplementary sources).

This study is being conducted collaboratively with scientists at the Wright State School of Medicine in Yellow Springs, Ohio. Data analysis is currently underway.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

A Mechanism for Carcinogen Resistance: Dietary Regulation of Carcinogen Efflux

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	G. C. Yeh	Senior Investigator	LNMR, CPRP, DCPC, NCI
Others:	J. M. Phang	Lab Chief	LNMR, CPRP, DCPC, NCI
	J. Lopaczynska	Visiting Fellow	LNMR, CPRP, DCPC, NCI
	M. Poore	Biolab Technician	LNMR, CPRP, DCPC, NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Nutritional and Molecular Regulation, CPRP, DCPC

SECTION

INSTITUTE AND LOCATION

NCI-Frederick Cancer Research and Development Center, Frederick, Maryland

TOTAL STAFF YEARS:

3.75

PROFESSIONAL:

2.75

OTHER:

1.00

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human Subjects
 ☐ (b) Human tissues
 ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

FORMER TITLE:

A New Mechanism for Carcinogen Resistance: Regulation by Diet and Nutrients

We previously demonstrated that chemical carcinogens, benzo(a)pyrene and 7, 12-dimethylbenzanthracene efflux mediated by the multidrug resistant (MDR) glycoprotein 170 (P-gp) in our tissue culture cells. The drugs and carcinogen efflux pump, P-gp may serve as an important mechanism in diet-dependent cancer prevention. We further demonstrated that diet-derived flavonols, galangin, quercetin, and kaempferol markedly stimulated the P-gp mediated efflux of benzo(a)pyrene, dimethylbenzanthracene, and adriamycin in human breast cancer MCF-7 and human colon cancer HCT-15 cells. Although P-gp, a product of the *mdr* gene, is known to play a critical role in cellular resistance to cytotoxic drugs, its function in normal cells has not been defined. We are investigating the mechanism and regulation of P-gp mediated carcinogen efflux by dietary factors in normal tissues as a functional mechanism of diet and cancer prevention.

We are examining nutrients and diet-derived constituents in modulating the functional role for increased P-gp expression. We showed that retinoic acid may serve as a substrate for P-gp and enhanced P-gp expression in our tissue culture cells but the functional role of retinoic acid or other related nutrients, e.g. β -carotene and carcinogen efflux mechanism are not clear. We propose that P-gp mediated carcinogen efflux is regulated by flavonoids, nutrients, and other dietary factors. These nutritional and molecular regulation of P-gp mediated carcinogen efflux mechanisms to cancer prevention are currently under investigation.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Nutritional Regulation of Carcinogens in Placenta-Related Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: C. A. Plouzek Senior Staff Fellow LNMR, CPRP, DCPC, NCI

Others: G. C. Yeh Senior Investigator LNMR, CPRP, DCPC, NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Nutritional and Molecular Regulation, CPRP, DCPC

SECTION

INSTITUTE AND LOCATION

NCI-Frederick Cancer Research and Development Center, Frederick, Maryland

TOTAL STAFF YEARS:

0.6

PROFESSIONAL:

0.6

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human Subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The plasma membrane glycoprotein 170 (P-gp), responsible for multidrug resistance, MDR, may also function as an efflux pump for chemical carcinogens and is regulated by dietary nutrients. P-gp is found in normal tissues, predominately in the cells lining the luminal space of a variety of tissues, including the placenta and the endometrium of the gravid uterus. Recently, our laboratory demonstrated that P-gp mediates the efflux of chemical carcinogens, benzo(a)pyrene and dimethylbenzanthracene, and we proposed that P-gp in normal tissues may serve as a first line of defense against carcinogens. In order to examine the expression of P-gp in normal cells, we used normal rat placental cells immortalized with SV40 temperature-sensitive A (*tsA*) mutant. We found at 33 degrees C (transformed phenotype) P-gp was not detectable but at 39.5 degrees C (normal differentiated phenotype) cells expressed significant amounts of P-gp protein as measured by Western immunoprecipitation with the monoclonal antibody, C219. Progesterone effectively blocks adriamycin efflux in the differentiated placental cells, as well as vinblastine accumulation, indicating that P-gp in placental cells may be regulated by progesterone. However, progesterone is not a substrate for P-gp since progesterone accumulation and efflux at 33 degrees C is similar to that at 39 degrees C. We further developed doxorubicin-resistant placental cells from these SV40-*tsA* rat placental cells and found a 2-4 fold increase of P-gp in doxorubicin-resistant placental cells at the differentiated phenotype. This is the first demonstration of normal differentiated placental cells with high expression of P-gp.

The functional role of P-gp in normal tissues has not been determined. However, our recent findings indicate that P-gp mRNA is developmentally regulated in human placental tissues. Currently, a developmental study in rats of P-gp expression in normal tissues during gestation is in progress. Rat placentas, ovaries, uteri, adrenals, kidneys, and colon mucosal cells at 0, 6, 9, 12, 15, and 18 days of gestation are being processed for examination of P-gp expression at the mRNA and protein levels.

In addition, we are also studying the regulation of P-gp in a human endometrial adenocarcinoma cell line and a human cervical carcinoma cell line. Dietary effectors such as retinoic acid (which is known to enhance P-gp) and the flavonoid, kaempferol (which decreases P-gp) are being investigated in the human endometrial and cervical cell lines to determine their effects. The role of *mdr1* and *mdr3* in the placental-related cell lines is under investigation to determine their expression under normal physiological conditions.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Effect of Proteins, Peptides, and Amino Acids on Carcinogenesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J. M. Phang Lab Chief LNMR, CPRP, DCPC, NCI
Others: G. C. Yeh Senior Investigator LNMR, CPRP, DCPC, NCI
J. P. Henry IRTA Fellow LNMR, CPRP, DCPC, NCI
S. J. Downing Chemist LNMR, CPRP, DCPC, NCI

COOPERATING UNITS (if any)

Johns Hopkins School of Medicine, Baltimore, MD (D. Valle)

LAB/BRANCH

Laboratory of Nutritional and Molecular Regulation, CPRP, DCPC

SECTION

INSTITUTE AND LOCATION

NCI-Frederick Cancer Research and Development Center, Frederick, Maryland

TOTAL STAFF YEARS:

2.4

PROFESSIONAL:

1.4

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human Subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Modulation of cellular signaling mechanisms by qualitative differences in dietary proteins and their metabolites were studied at the level of:

- Imidodipeptides,
- Pyrroline 5-carboxylate reductase as a mediator of redox exchange, and
- Effect of pyrroline 5-carboxylate on mitogenesis-effect on membrane phosphoinositides

a. Imidodipeptides. Dipeptides containing proline or hydroxyproline originate from either tissue matrix degradation or from protein nutrition. They circulate in plasma and are delivered to tissues where they are hydrolyzed by prolidase. Thus, prolidase is a potential interface between protein nutrition and matrix breakdown. Our studies showed that the level of cellular prolidase is regulated by extracellular collagen acting through integrin receptors. Thus, the hydrolysis of imidodipeptides, the final degradative products of matrix collagen, is responsive to cellular interaction with extracellular matrix. We are studying the regulation of this enzyme on the molecular level.

b. Pyrroline 5-carboxylate reductase. We are studying this enzyme, which catalyzes the committed step in proline biosynthesis, on the molecular level. Previous studies suggested that it also functions in plasma membrane redox transfers. Using Western blots to analyze cellular fractions, we have shown that the enzyme is associated physically with cellular plasma membranes. The molecular mechanisms for this association is being investigated.

c. Effect of pyrroline 5-carboxylate on mitogenesis. P5C stimulates PRPP and purine ribonucleotide synthesis synergistically with platelet-derived growth factor. It also increases the incorporation of thymidine in serum-activated cells. Inhibitor studies suggest that the effect is due to the turnover of membrane phosphoinositides, and direct assays show that it is phospholipase D which is stimulated by P5C with the release of phosphatidic acid. Possible interaction of this system with various growth factors and its modulation of intermediate metabolism are being pursued.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Nitric Oxide, Dietary Factors, and Signal Transduction

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J. M. Phang

Lab Chief

LNMR, CPRP, DCPC, NCI

Others: J. M. Mei

Scientist

Program Resources, Inc.

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Nutritional and Molecular Regulation, CPRP, DCPC

SECTION

INSTITUTE AND LOCATION

NCI-Frederick Cancer Research and Development Center, Frederick, Maryland

TOTAL STAFF YEARS:

0.5

PROFESSIONAL:

0.4

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human Subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

FORMER TITLE: Dietary Lipids and Signal Transduction in Breast Cells

Reducing-oxidizing reactions function not only in immune defense mechanisms but also in cellular signaling and regulation. Genotoxic events mediated by oxidants and the protection of antioxidants have been suggested in carcinogenesis and in the mechanisms of diet-dependent cancer prevention. In this context, nitric oxide may play an important role. Enzymatically synthesized by nitric oxide synthase, this molecule can form other reactive oxygen species and act in both pro-oxidant and antioxidant pathways. Therefore, the level of this enzyme and its regulation by dietary compounds and nutrients is of interest for carcinogenesis and cancer prevention. The inducible form of nitric oxide synthase (iNOS) is found in macrophages and can be induced by lipopolysaccharide and interferon gamma. We used two methods for estimating enzyme activity. First, in a direct cell-free assay system, the production of citrulline from precursor radiolabeled arginine was quantitated by recovering citrulline with ion-exchange chromatography. Second, an estimate of activity in intact cells was based on the accumulation of nitrite in tissue culture medium. Using these methods, we have studied several tissue culture cells using a variety of nutrients, cytokines, pro-oxidant and antioxidant effector molecules in order to identify interactions of dietary compounds and nutrients with this system of potential importance in carcinogenesis. After identifying regulatory effectors, we will use Western immunoblotting to characterize the enzyme protein. We will develop a cDNA probe to study the expression of iNOS and its regulation by dietary and nutritional factors.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Nutritional Modulation of Apoptosis: Regulation of Oncoproteins and Suppressors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: T. Wang Senior Staff Fellow LNMR, CPRP, DCPC, NCI

Other: J. M. Phang Lab Chief LNMR, CPRP, DCPC, NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Nutritional and Molecular Regulation, CPRP, DCPC

SECTION

INSTITUTE AND LOCATION

NCI-Frederick Cancer Research and Development Center, Frederick, Maryland

TOTAL STAFF YEARS:

0.7

PROFESSIONAL:

0.7

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human Subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

FORMER TITLE: Regulation of Tumor Suppressor Protein p53

Apoptosis is a normal physiological process by which multicellular organisms maintain cell number. Deregulation of apoptosis may be involved in tumorigenesis. It is known that tumor suppressor protein p53, oncoprotein bcl-2, bax, myc, myb, and Apo1/Fas are components of the apoptotic pathway. In the immune system, apoptotic signals can be triggered by steroid hormones and cytokines. However, little is known about the apoptotic signals and the molecular mechanisms in tissues of epithelial origin such as gastrointestinal tract and breast. Cell proliferation and differentiation are modulated not only by steroid hormones and cytokines but also by dietary compounds such as flavanoids and retinoids. Furthermore, dietary manipulation such as caloric restriction modulates cellular proliferation either directly or indirectly by altering the hormonal status of experimental animals. Thus, it is possible that dietary or nutritional factors may lead to modulation of apoptosis. We are interested in exploring dietary and nutritional effects on apoptosis. Our initial focus will be on modulation of the apoptosis related proteins. The apoptosis related proteins p53, bcl-2, bax, myc, myb, and Apo1 will be examined at the transcriptional, translational, and posttranslational level to elucidate possible effects. In addition, we will develop cell culture system(s) that will allow us to identify dietary factors which modulate apoptosis. We will also explore the process of apoptosis at the whole animal level. Genetically engineered p53-knockout mice will be used to study the relationship between p53 and other apoptosis related proteins. In addition, dietary manipulation of p53-knockout mice may reveal effects of dietary factors on apoptosis.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Nutritional Regulation of *Ras* Proto-Oncogene Activity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)

PI: S. N. Perkins Senior Staff Fellow LNMR, CPRP, DCPC, NCI

Others: J. M. Phang Lab Chief LNMR, CPRP, DCPC, NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Nutritional and Molecular Regulation, CPRP, DCPC

SECTION

INSTITUTE AND LOCATION

NCI-Frederick Cancer Research and Development Center, Frederick, Maryland

TOTAL STAFF YEARS:

0.8

PROFESSIONAL:

0.8

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human Subjects ☐ (b) Human tissues ☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Ras-mediated escape from normal regulation appears to be a frequent event in the multi-step genesis of cancer. A number of *in vitro* studies have demonstrated interactions between *ras* and other proto-oncogene products, especially *myc* and the tumor suppressor p53. A useful model for the study of these interactions is the p53 "knockout" mouse, a transgenic mouse in which null p53 germ line mutations prevent the expression of either one or both alleles for p53. Such p53-deficient animals develop normally but are prone to early tumorigenesis. We are using these mice both to study the effects of various diets and p53 gene dosage on carcinogenesis, and as a model of accelerated carcinogenesis that does not require exposure to chemicals that initiate or promote tumorigenesis. Moreover, fibroblasts cultured from embryos with the various genetic backgrounds afford a powerful *in vitro* system for addressing some of the same issues. We are using probes for *ras*, *myc*, and p53 to assess the expression of these proto-oncogenes in various tissues from transgenic mice.

Also of special interest is a recently described mediator of many p53 actions, *WAF1/Cip1*. With the polymerase chain reaction (PCR), we have amplified and cloned a portion of the mouse *WAF1* sequence and are using it as a probe to examine the role of this novel protein in p53-deficient and wildtype mice that have undergone various dietary manipulations.

Studies in progress with Dr. S. Hursting are investigating the effects of two potent but poorly understood dietary regimens that dramatically delay tumor development in rodents: calorie restriction and supplementation with dehydroepiandrosterone and related steroids. These studies will determine how such dietary manipulations combine with the gene dosage of p53 to affect proto-oncogene expression and the activity of certain key metabolic enzymes.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Fiber and Phytohormones in Cancer Prevention

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: N. Sathyamoorthy Senior Staff Fellow LNMR, CPRP, DCPC, NCI

Other: J. M. Phang Lab Chief LNMR, CPRP, DCPC, NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Nutritional and Molecular Regulation, CPRP, DCPC

SECTION

INSTITUTE AND LOCATION

NCI-Frederick Cancer Research and Development Center, Frederick, Maryland

TOTAL STAFF YEARS:

0.6

PROFESSIONAL:

0.6

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human Subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

FORMER TITLE: Role of Fiber and Hormone-Like Compounds in Cancer Prevention

We have developed a sensitive, rapid and non-radioactive *in vitro* assay to screen diet-derived compounds for estrogen-like activity by monitoring the expression of pS2 mRNA in MCF-7 human breast cancer cells. Using our method we successfully identified several compounds, including NDGA, daidzein, genistein, equol, and kaempferol as being estrogenic. Our results were recently published in Cancer Research (Vol. 54, 1994).

The isoflavones daidzein and genistein are major components of soy food; equol is derived from daidzein by the action of gut microflora. Epidemiological evidence indicates that increased consumption of soy foods is associated with lowered risk for hormone dependent cancers. It has been postulated that these compounds contribute to beneficial effects of soy food. We were interested in studying the molecular mechanism by which they exert their effects. We examined the ability of the various compounds to compete with estradiol for binding to the estrogen receptor. The estrogenic compounds like daidzein, equol, and genistein were also very effective in displacing labeled estradiol from binding to the estrogen receptor. The expression of estrogen receptor mRNA in MCF-7 cells in response to long term exposure to genistein was also evaluated. We have two manuscripts ready for submission based on this work.

We are currently studying the effect of the isoflavones on the expression of TGF- β levels in MCF-7 cells using a Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) method.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Effects of Vitamin A Nutriture and Synthetic Retinoids on Retinol Metabolism

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	K. C. Lewis	Senior Staff Fellow	LNMR, CPRP, DCPC, NCI
Others:	J. M. Phang	Lab Chief	LNMR, CPRP, DCPC, NCI
	L. A. Zech	Senior Scientist	LMMB, DCBDC, NCI

COOPERATING UNITS (if any)

Laboratory of Mathematical Biology

LAB/BRANCH

Laboratory of Nutritional and Molecular Regulation, CPRP, DCPC

SECTION

INSTITUTE AND LOCATION

NCI-Frederick Cancer Research and Development Center, Frederick, Maryland

TOTAL STAFF YEARS:

1.2

PROFESSIONAL:

1.2

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human Subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The clinical usefulness of retinoids is not likely to be fully realized until basic aspects of their metabolism are better understood. Accordingly, the general focus in our laboratory in regard to our work with retinoids has been twofold: 1) to investigate the various mechanisms involved in the normal physiological metabolism of vitamin A at the molecular, tissue, and whole body level and 2) to examine how the administration of certain retinoids shown to be clinically useful in regard to cancer treatment or prevention affect normal vitamin A metabolism.

Several studies have been completed, including two long-term experiments involving the administration of either N-[4-hydroxyphenyl] retinamide (4-HPR) or all-*trans* retinoic acid (RA). Tracer kinetic studies were carried out in these experiments, and various kinetic parameters were determined using the SAAM/CONSAM computer modelling programs. Analysis of data indicated that these compounds significantly perturbed normal vitamin A metabolism. Both retinoids reduced normal plasma vitamin A levels and altered plasma kinetics of the vitamin. Additionally, these compounds had variable effects on individual tissue levels and kinetics of the vitamin A. For example, as compared to the control, the fraction of the plasma retinol being catabolized per day was nearly twice as high in the CON + 4-HPR treated group, whereas it was nearly the same in the retinoic acid-treated group. Before being lost from the system, a retinol molecule, on average, recycled through the plasma of the 4-HPR treated group only about half the number of times as it did in the control group; however, in contrast, the RA-treated group recycled a molecule of the vitamin nearly twice as many times as compared to the control.

The possible consequences of these and other such alterations of vitamin A metabolism remain to be clarified. We are investigating potential mechanisms by which 4-HPR and RA alters retinol metabolism. Thus, we have developed molecular probes to determine whether an altered expression of certain retinoid binding proteins might be involved. Our initial findings indicate that in the tissues examined thus far (liver and kidney), the expression of retinol-binding protein or the cellular binding proteins for retinol or retinoic acid does not appear to be affected. We are presently developing compartmental models that will more fully describe vitamin A metabolism in individual organs as well as whole body metabolism of the vitamin. Additionally, we have expanded our studies at the molecular level and have initiated a number of tissue culture studies in relevant tumor cell lines. Whether or not the 4-HPR and RA associated alterations in vitamin A metabolism we have observed in an animal model might also occur in humans with possible untoward clinical consequences deserves careful evaluation.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanisms for Deranged Androgen Response in Prostate Cancer Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J. M. Phang Lab Chief LNMN, CPRP, DCPC, NCI

Others: X. Y. Sun IRTA Fellow LNMN, CPRP, DCPC, NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Nutritional and Molecular Regulation, CPRP, DCPC

SECTION

INSTITUTE AND LOCATION

NCI-Frederick Cancer Research and Development Center, Frederick, Maryland

TOTAL STAFF YEARS:

1.1

PROFESSIONAL:

1.1

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human Subjects ☐ (b) Human tissues ☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Androgens (testosterone and its metabolites) play a large role in the normal growth and function of the prostate. However, changes in androgen metabolism or responsiveness to androgens have been implicated in the formation of benign prostatic hypertrophy and prostate cancer. The causes of these changes are not well understood. Studies were undertaken to determine what, if any, differences in androgen metabolism occur between androgen dependent and androgen independent prostate cancer cells.

In whole cell studies, we showed that added testosterone is primarily glucuronidated. Prostate cancer cells which are active in metabolizing testosterone to testosterone glucuronide were shown by assays with cell-free extracts to have UDP-glucuronyl transferase activity. This enzyme has been considered primarily to be hepatic and has not been emphasized in the prostate. Therefore the enzyme may be an important determinant of androgen responsiveness in the prostate and its regulation by dietary compounds or nutrients was of interest.

We found that certain flavonoids are active in increasing UDP-glucuronyl transferase activity. Genistein and biochanin A were especially active, with the latter increasing the activity by 6 to 7-fold. In studies using Michaelis-Menten kinetics, we found the activity in stimulated cells had affinity for testosterone identical to activity from unstimulated cells. Therefore, induction of enzyme synthesis was likely. We are characterizing the induction of UDP-glucuronyl transferase in these cells at the RNA and protein levels.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Molecular Mechanisms of Oncogene Action

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M. J. Birrer	Section Chief	BPRB, EDCOP, DCPC, NCI
Others:	P. H. Brown	Senior Investigator	BPRB, EDCOP, DCPC, NCI
	A. Sabichi	Clinical Associate	BPRB, EDCOP, DCPC, NCI
	L. Nader	Biologist	BPRB, EDCOP, DCPC, NCI
	T. Chen	Biologist	BPRB, EDCOP, DCPC, NCI
	S. Kim	Fogarty Fellow	BPRB, EDCOP, DCPC, NCI

COOPERATING UNITS (if any)

Division of Cancer Etiology, NCI (N. Colburn)
University of Rochester (D. McCance)
University of California at San Diego (M. Karin)
Johns Hopkins University (C. Dang)

LAB/BRANCH

Biomarkers and Prevention Research Branch, EDCOP, DCPC

SECTION

Molecular Mechanisms Section

INSTITUTE AND LOCATION

National Cancer Institute, Rockville, Maryland

TOTAL STAFF YEARS:

3.0

PROFESSIONAL:

3.0

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human Subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Recent developments in the application of molecular biology to epithelial cancers have led to the identification of specific genetic lesions resulting in either activation or inactivation of key target genes. These genes, called oncogenes, are involved in various aspects of cell growth regulation and as such play major roles in the early carcinogenic processes of "initiation" and "promotion." It is now critical to understand the precise mechanisms by which these genes function so molecular or pharmacologic agents can ultimately be derived to alter or repress their effects.

The purpose of this project is to elucidate the biochemical and molecular mechanisms by which oncogenes transform mammalian cells. To this end, we have performed structure/function analysis on members of the *myc*, *jun* and *fos* oncogene families. These studies have revealed various structural aspects of these proteins which are necessary and sufficient for transformation.

Our studies of the *c-jun* oncogene revealed that in addition to the DNA binding and dimerization domains, the N-terminal transactivation domain is required for cellular transformation. In addition, the ability of *c-jun* to transactivate correlates with its ability to transform cells. Thus, *c-jun* appears to transform cells by regulating gene expression. Further, detailed mutation analysis of *c-jun* has demonstrated that phosphorylation of cJun at serines 63/73 results in increased transactivation and ultimately transformation. The phosphorylation of these sites occurs in part through a *ras/raf* dependent pathway which provides an important biochemical link between these oncogenes. More recent studies are aimed at a more detailed analysis on other *c-jun* post-translational modifications and their biochemical and biologic effects and parallel studies with the *c-fos* oncogene examining the relationship between phosphorylation and biologic activity. This work has revealed that small amino acid deletion in the N-terminus transactivation domain results in constitutive phosphorylation of *c-jun* and increased transactivation.

Our studies of the *myc* oncogene have focused on comparing the transactivating and transforming activities of the *c-myc* and *L-myc* genes. By exon shuffling, we have demonstrated that *L-myc* transactivates and transforms much less efficiently than *c-myc* and this difference is localized to the second exon. More recent work has focused on the precise structural differences between these genes and their role in apoptosis.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Use of Transcriptional Factors as Targets and Agents for Chemoprevention

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M. J. Birrer	Section Chief	BPRB, EDCOP, DCPC, NCI
Others:	P. H. Brown	Senior Investigator	BPRB, EDCOP, DCPC, NCI
	A. Sabichi	Clinical Associate	BPRB, EDCOP, DCPC, NCI
	L. Nader	Biologist	BPRB, EDCOP, DCPC, NCI
	T. Chen	Biologist	BPRB, EDCOP, DCPC, NCI
	S. Kim	Fogarty Fellow	BPRB, EDCOP, DCPC, NCI

COOPERATING UNITS (if any)

University of Arizona (G. T. Bowden)
Johns Hopkins University (C. Dang)
University of California at San Diego (M. Karin)
Division of Cancer Etiology, NCI (N. Colburn and U. Rapp)

LAB/BRANCH

Biomarkers and Prevention Research Branch, EDCOP, DCPC

SECTION

Molecular Mechanisms Section

INSTITUTE AND LOCATION

National Cancer Institute, Rockville, Maryland

TOTAL STAFF YEARS:

3.0

PROFESSIONAL:

3.0

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human Subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Transcription factors are critical regulators of gene expression. It is clear that these factors control the expression of many genes and, as such, mediate the biologic effects of agents such as "tumor promoters." The purpose of this project is to design mutants of transcription factors specifically aimed at inhibiting their biochemical and most importantly their biologic functions.

The AP-1 complex has been specifically implicated in mediating the biologic effects of the tumor promoters "phorbol esters." A major component of this complex is the *c-jun* oncogene. We have created and tested a series of dominant-negative mutants of *c-jun* which are able to inhibit the biochemical functions of this oncogene. A transactivation mutant with a deletion of the N-terminal amino acids 2-122 has been shown to inhibit AP-1 transactivation and *c-jun* transformation. In addition, this mutant has been shown to inhibit cellular transformation by a wide range of oncogenes including *c-fos*, *c-raf*, *ras*, *mos*, and *myc*. Further, stable expression of this mutant protein in mouse epidermal cells can block phorbol ester induced tumor promotion. The mechanism of action of this mutant has been demonstrated to be heterodimerization with and neutralization of other AP-1 complex components such as *c-fos*.

Our most recent efforts are aimed at furthering refining the potency and specificity of these mutants by creating smaller mutants with higher affinities for dimerization and DNA binding and testing them in specific human tumor systems such as breast and lung cancers. We have now created a series of *c-jun* mutants containing larger N-terminal deletions producing small peptides containing only the leucine zipper (dimerization domain). In addition, three DNA binding mutants including one with a point mutation at position 265, a deletion at positions 269-272, and one with an insertion of 3 amino acids at position 265 have been produced. All of these mutants have been demonstrated to have a dominant-negative phenotype.

Future efforts are aimed at designing delivery mechanisms which might make these agents more clinically applicable. *In vivo* testing will be performed in model systems. Finally, we will expand these studies to include other transcription factors which play critical roles in human carcinogenesis such as CREB.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Expression of TGF- β Isoforms in Human Lung Cancer Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: S. B. Jakowlew

Senior Investigator

BPRB, EDCOP, DCPC, NCI

Others: T. W. Moody

Section Chief

BPRB, EDCOP, DCPC, NCI

A. Mathias

Technician

BPRB, EDCOP, DCPC, NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Biomarkers and Prevention Research Branch, EDCOP, DCPC

SECTION

Experimental Biochemistry Section

INSTITUTE AND LOCATION

National Cancer Institute, Rockville, Maryland

TOTAL STAFF YEARS:

1.6

PROFESSIONAL:

1.5

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human Subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The transforming growth factor-betas (TGF- β s) are a group of structurally related peptides that exert multiple effects in various cell types. For example, TGF- β acting as a multifunctional growth regulatory molecule either stimulates or inhibits the growth of mesenchymal or epithelial cells, respectively. Expression of TGF- β 1, 2 and 3 ligands and TGF- β type I, II and III receptors was examined in cultured human lung cancer cells. Specific cDNA probes and antibodies for TGF- β s 1, 2 and 3 were used to study expression of these different TGF- β isoforms in both non-small cell lung cancer cells (NSCLC) and small cell lung cancer cells (SCLC). Expression of TGF- β 1 mRNA was detected in both cell types using Northern blot hybridization, with expression being significantly higher in NSCLC cells. Furthermore, expression of TGF- β 2 and TGF- β 3 mRNAs was also detected in NSCLC and SCLC cells. The relative levels of expression of the TGF- β mRNAs was TGF- β 1 > TGF- β 2 > TGF- β 3. Expression of TGF- β type I, II and III receptor mRNAs was also detected in both NSCLC and SCLC cells, with expression of these mRNAs being higher in NSCLC cells than in SCLC cells. The relative level of expression of the TGF- β receptor mRNAs was type I > type II > type III. TGF- β 1 protein was detected in the conditioned medium of NSCLC and SCLC cells, with the level being higher in several NSCLC cells than in SCLC cells. Addition of TGF- β resulted in an increase in the level of TGF- β 1 mRNA and a corresponding increase in the amount of TGF- β 1 protein in some NSCLC cells. Our results demonstrate co-expression of the TGF- β isoforms and their receptors in human NSCLC cells, with expression of TGF- β 1 mRNA and protein more prominent than that of TGF- β s 2 and 3 and TGF- β type I receptor mRNA more prominent than that of TGF- β type II and III receptors.

Expression of retinoic acid receptor (RAR) and retinoid X receptor (RXR) mRNAs was also detected in both NSCLC and SCLC cells. The level of expression of RAR- α , RAR- β and RAR- γ mRNAs was approximately equal in most NSCLC cells, while expression of RAR- α mRNA was equal to or greater than that of RAR- β mRNA and significantly higher than that of RAR- γ mRNA in most SCLC cells. Expression of RXR- α , RXR- β and RXR- γ mRNAs was approximately equal in both NSCLC and SCLC cells. Retinoic acid increased expression of TGF- β 2 mRNA and decreased expression of TGF- β 3 mRNA in some NSCLC cells; retinoic acid had no effect on TGF- β mRNAs in SCLC cells. Retinoic acid significantly inhibited colony formation of several NSCLC cells.

The significance of the project is to increase the expression of one or more of the TGF- β isoforms in lung cancer cells by treatment with chemopreventive agents such as retinoic acid. Increased TGF- β production may be used to slow the proliferation of lung cancer cells.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cellular Differentiation in Normal and Neoplastic Respiratory Epithelium

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R. I. Linnoila	Section Chief	BPRB, EDCOP, DCPC, NCI
Others:	E. Szabo	Senior Investigator	BPRB, EDCOP, DCPC, NCI
	J. E. Jones	Senior Staff Fellow	BPRB, EDCOP, DCPC, NCI
	S. M. Jensen	Biologist	BPRB, EDCOP, DCPC, NCI
	T. A. T. Bunnag	Biologist	BPRB, EDCOP, DCPC, NCI
	M. Sagawa	Visiting Fellow	BPRB, EDCOP, DCPC, NCI
	M. Ebina	Visiting Fellow	BPRB, EDCOP, DCPC, NCI

COOPERATING UNITS (if any)

NCI-Navy Medical Oncology Branch, DCT, NCI (H. Oie and B. E. Johnson)
Surgery Branch, DCT, NCI (H. Pass and S. Steinberg)
University of California, Davis (H. Witschi)

LAB/BRANCH

Biomarkers and Prevention Research Branch, EDCOP, DCPC

SECTION

Experimental Pathology Section

INSTITUTE AND LOCATION

National Cancer Institute, Rockville, Maryland

TOTAL STAFF YEARS:

6.0

PROFESSIONAL:

3.0

OTHER:

3.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human Subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our aim is to characterize the cellular differentiation and genetic damage associated with premalignant changes in respiratory epithelium. This has been studied at the level of:

A. Peripheral airway cell differentiation. We found 30% of the 400 non-small cell lung carcinomas (NSCLC) examined to be positive for at least one of the peripheral airway cell (PAC) markers SP-A and CC10. They also formed a clinically distinct subset. Characterization of NSCLC cell lines expressing PAC markers is in progress. In order to define premalignant lesions, we are studying the response of PACs in non-neoplastic lung to pulmonary carcinogens, including tobacco specific nitrosamines.

B. Clara cell specific protein (CC10) also known as a PCB (a potent carcinogen)-binding protein. We have demonstrated that nonciliated secretory cells, which are progenitor cells for the epithelium and NSCLC, express high levels of CC10, while only 10% of NSCLC are positive for CC10. Our preliminary results showed that in the presence of smoking related atypia the patterns of CC10 mRNA expression in non-neoplastic human lung were affected both in larger airways and alveoli, while changes in smaller airways were minimal. Changes involved both intensity and cellular distribution of mRNA. Further studies are in progress.

C. Neuroendocrine differentiation. We have demonstrated that 15% of NSCLC tumors express multiple neuroendocrine (NE) features. Our results indicate that these tumors are sensitive to chemotherapy. The role of NE differentiation in non-neoplastic epithelium is investigated.

D. Expression of oncogenes and tumor suppressor genes. We have found overexpression of *c-myc* in a high number of NSCLC as well as in the progenitor cells in human lung by in situ hybridization. In a cohort of 120 NSCLC patients, overexpression of p53 tumor suppressor gene was correlated with shorter survival in a subset of patients. Molecular analysis of the potentially prognostic mutations, and the mutations in premalignant changes in the surrounding non-neoplastic lung is in progress. These mutations will be correlated with *ras* and chromosome 3p abnormalities.

The significance of the project is that the results will provide a rational basis for innovative approaches for early detection and intervention in human lung cancer.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

CYP1A1 Gene Regulation and Human Cancer

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)

PI:	J. E. Jones	Senior Staff Fellow	BPRB, EDCOP, DCPC, NCI
Others:	R. I. Linnoila	Section Chief	BPRB, EDCOP, DCPC, NCI
	S. M. Jensen	Biologist	BPRB, EDCOP, DCPC, NCI
	E. Unsworth	Chemist	BPRB, EDCOP, DCPC, NCI
	G. Shaw	Senior Investigator	BPRB, EDCOP, DCPC, NCI

COOPERATING UNITS (if any)

NCI-Navy Medical Oncology Branch, DCT, NCI (H. Oie)
Surgery Branch, DCT, NCI (H. Pass)
Johns Hopkins University School of Hygiene and Public Health (G. Peterson)

LAB/BRANCH

Biomarkers and Prevention Research Branch, EDCOP, DCPC

SECTION

Experimental Pathology Section

INSTITUTE AND LOCATION

National Cancer Institute, Rockville, Maryland

TOTAL STAFF YEARS:

3.4

PROFESSIONAL:

2.4

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human Subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

A major goal of our laboratory has been the elucidation of the role of the human cytochrome P450, *CYP1A1*, in lung carcinogenesis. The protein product of the *CYP1A1* gene and its associated catalytic activities, including aryl (or aromatic) hydrocarbon hydroxylase (AHH), are known to be intimately associated with the metabolic activation of many of the procarcinogenic polycyclic aromatic hydrocarbons (PAHs) found in cigarette smoke and other environmental pollutants to highly reactive intermediates. Elevated levels of AHH activity have been implicated in numerous studies as a significant risk factor in the etiology of lung cancer. Our investigations have been focused upon elucidating the mechanisms by which *CYP1A1* gene expression is regulated and to establish a functional relationship between deviations from normal patterns of expression and lung cancer.

- A. Our studies of the regulation of expression of the human *CYP1A1* gene using oligonucleotide directed mutagenesis and reporter gene expression in non-small cell lung cancer derived cell lines reveal that transcriptional activation of the gene is mediated through two widely separated DNA regulatory elements. This activation appears to be synergistic, as each element contributes roughly only 30% to the overall expression of the gene. These data are supported by gel mobility shift analyses of protein/DNA interactions at each site under control and inducing conditions. This work represents the first effort at characterization of the regulatory elements of the human *CYP1A1* gene and the determination of the role for each in the regulation of expression of the gene in the adult lung.
- B. Interindividual variability in *CYP1A1* gene expression may arise as a result of genetic differences within the genes encoding *CYP1A1* transcriptional regulatory proteins. We have identified at least two restriction fragment length polymorphisms (RFLPs) within the gene encoding the major transcriptional activator of the *CYP1A1* gene, the aromatic hydrocarbon (Ah) receptor. DNA from age, race, and sex matched control and histologically confirmed lung cancer patients is presently under examination to determine the frequency of these newly acquired genetic markers within the two populations.

The significance of these projects is the elucidation of the interactive role of genetically determined factors and chemical carcinogens in pulmonary carcinogenesis. The results will have diagnostic and prevention applications.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Rational Applications of Biomarkers in Clinical Trials

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	G. L. Shaw	Senior Investigator	BPRB, EDCOP, DCPC, NCI
Others:	M. J. Birrer	Section Chief	BPRB, EDCOP, DCPC, NCI
	S. Jakowlew	Senior Investigator	BPRB, EDCOP, DCPC, NCI
	M. Schiffman	Physician Epidemiologist	EES, EEB, EBP, DCE, NCI
	S. Lemon	Cancer Prevention Fellow	BPRB, EDCOP, DCPC, NCI

COOPERATING UNITS (if any)

National Naval Medical Center (B. Ghosh and W. Laskin)

LAB/BRANCH

Biomarkers and Prevention Research Branch, EDCOP, DCPC

SECTION

Experimental Intervention Section

INSTITUTE AND LOCATION

National Cancer Institute, Rockville, Maryland

TOTAL STAFF YEARS:

0.5

PROFESSIONAL:

0.5

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human Subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

FORMER TITLE: Rational Applications of Biomarkers in Colon Cancer

A major challenge for the BPRB is to begin to apply specific biomarkers in a rational way to permit more effective early detection approaches. Our laboratory resources will permit an intensive characterization of biomarkers and the biologic effects of intervention agents in a pilot study setting. Multiple markers have been implicated in the pathogenesis of human malignancies. Point mutations in the *ras* oncogene have been identified in colorectal and lung carcinomas and have recently been identified in shed epithelial cells found in stool specimens from patients with colorectal carcinomas. Alterations in carbohydrate antigen expression have also been found in malignancies and may be useful markers of neoplastic change.

Shed epithelial cells in archived stool specimens from patients with documented colorectal cancer will be analyzed using polymerase chain reaction for oncogene mutations or activation, or changes in carbohydrate antigen expression. These findings will be correlated with the markers present in the archived surgical material. Blocks from patients entered on a case-control study of colorectal cancer conducted at the National Naval Medical Center, Bethesda, Maryland have been obtained and are being analyzed for the presence of *ras* mutations and p53 expression. They will also be examined for carbohydrate antigen expression. If preliminary results are promising, additional specimens from patients entered on this study at Walter Reed Army Hospital and George Washington University Hospital, Washington, DC will also be obtained. Assays on stool specimens from control subjects will also be performed to assess the usefulness of these markers to discriminate patients with colorectal cancer from controls without cancer. The information obtained will be coupled with the previously collected epidemiologic data and Tumor Registry data for survival information to determine the potential usefulness for screening or prognostic purposes.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical Evaluation of New Intervention Agents

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J. L. Mulshine	Branch Chief	BPRB, EDCOP, DCPC, NCI
Others:	G. Shaw	Senior Investigator	BPRB, EDCOP, DCPC, NCI
	I. Avis	Biologist	BPRB, EDCOP, DCPC, NCI
	F. Cuttitta	Deputy Branch Chief	BPRB, EDCOP, DCPC, NCI
	T. Moody	Section Chief	BPRB, EDCOP, DCPC, NCI

COOPERATING UNITS (if any)

Biomeasure, Inc. (J. P. Moreau)
Nuclear Medicine Department, NIH Clinical Center (J. Carrasquillo)
Walter Reed Research Institute (M. Jett)

LAB/BRANCH

Biomarkers and Prevention Research Branch, EDCOP, DCPC

SECTION

Experimental Intervention Section

INSTITUTE AND LOCATION

National Cancer Institute, Rockville, Maryland

TOTAL STAFF YEARS:

1.5

PROFESSIONAL:

1.0

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human Subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Mlnors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We and others have demonstrated the role of gastrin releasing peptides (GRP) as an autocrine growth factor, and the weight of this evidence is consistent with GRP playing an important role in early cancer formation. We have evaluated the use of a neutralizing monoclonal antibody to block the effect of this growth factor in patients with advanced small cell lung cancer. This treatment is associated with no demonstrable toxicity, but only one patient had a significant anti-tumor response.

Based on this experience, we proposed to evaluate a new class of GRP antagonists that are synthetic peptides. This class of molecules may have better properties such as bioavailability and affinity than monoclonal antibodies. These molecules might also be tagged with a radioisotope to permit exact pharmacologic analysis. Synthetic peptide growth factor antagonists may be very useful for delivery as intervention agents, and we proposed to evaluate that possibility. This effort would be a model for the type of rational intervention agent research that the BPRB staff will conduct.

Ongoing efforts have included continuation of the Phase II monoclonal antibody trial with the NCI-Navy Medical Oncology Branch. BPRB staff is helping to produce a new preparation of the pharmaceutical grade antibody so the Phase II trial can be completed. Other approaches to blocking GRP dependent growth stimulation are also being developed using either amidating enzyme inhibitors or signal transduction inhibitors.

GRP is a molecule that serves as an excellent model of a neuropeptide effector that may be important as a mediator of tumor promotion dynamics in certain epithelium; as such it comprises an attractive target for intervention research.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CN 00171-03 BPRB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biological Regulation of Lung Cancer Growth

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J. L. Mulshine	Branch Chief	BPRB, EDCOP, DCPC, NCI
Others:	F. Cuttitta	Deputy Branch Chief	BPRB, EDCOP, DCPC, NCI
	T. Treston	Senior Investigator	BPRB, EDCOP, DCPC, NCI
	I. Avis	Biologist	BPRB, EDCOP, DCPC, NCI
	T. Moody	Section Chief	BPRB, EDCOP, DCPC, NCI

COOPERATING UNITS (if any)

Walter Reed Research Institute (M. Jett)

LAB/BRANCH

Biomarkers and Prevention Research Branch, EDCOP, DCPC

SECTION

Experimental Intervention Section

INSTITUTE AND LOCATION

National Cancer Institute, Rockville, Maryland

TOTAL STAFF YEARS:

0.95

PROFESSIONAL:

0.75

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human Subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have evaluated a number of compounds that influence the growth of lung cancer cells. We have reported previously on the autocrine role of gastrin release peptide, insulin-like growth factor, and transferrin--all of which stimulate growth for certain types of lung cancer. We have also shown that regulatory molecules such as glucagon and 13-*cis*-retinoic acid can inhibit the growth of a number of lung cancer cells lines. This experience has allowed us to focus on the signal transduction pathways most central to the process of cellular proliferation. In collaboration with Dr. M. Jett, we have recently presented data suggesting that 5-HETE, a product of 5-lipoxygenase activation may be a key intermediary in growth factor mediated growth stimulation of cancer cells. Since considerable information exists about the lipoxygenase pathway, we can potentially exploit the availability of existence of specific antagonists for application as biointervention tools. Recent work has extended the evaluation of the lipoxygenase inhibitors to other epithelial cancers, and most other types of cancers can be inhibited to various degrees. Systematic evaluation of the growth factor biology of early cancer cells may yield additional clues for the development of rational cancer intervention agents. Promising leads from *in vitro* models demonstrating significant anti-cancer effects with lung cancer cell lines will be followed up with evaluation of efficiency for *in vivo* model systems.

The most interesting *in vitro* leads will be evaluated for clinical application in Phase I and II studies conducted by the BPRB. An important part of that effort would be the identification of markers for intermediate end points analysis; these would accelerate the process of determining the benefit of this class of intervention tools.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunocytochemical Test for Early Lung Cancer Detection CRADA# 35516

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J. L. Mulshine	Branch Chief	BPRB, EDCOP, DCPC, NCI
Others:	F. Scott	Guest Researcher	BPRB, EDCOP, DCPC, NCI
	I. Avis	Biologist	BPRB, EDCOP, DCPC, NCI
	M. S. Tockman	Associate Professor	Johns Hopkins
	P. Gupta	Professor	University of Pennsylvania
	J. Tomita	Group Leader	Abbott Laboratories

COOPERATING UNITS (if any)

Johns Hopkins University, Abbott Laboratories, University of Pennsylvania, Illinois Cancer Council, Memorial Sloan Kettering Institute, University of Toronto, University of South Florida, University of Colorado, University of Texas at San Antonio, M.D. Anderson, Quebec Cancer Centre

LAB/BRANCH

Biomarkers and Prevention Research Branch, EDCOP, DCPC

SECTION

Experimental Intervention Section

INSTITUTE AND LOCATION

National Cancer Institute, Rockville, Maryland

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

0.25

OTHER:

0.75

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human Subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

We have established a CRADA to prospectively validate the diagnostic accuracy of a lung cancer early detection approach. A clinical team of investigators from 11 institutions throughout the United States and Canada is accruing stage I resected lung cancer patients to a protocol where annual induced sputums will be acquired and immunostained. Ongoing patient followup will permit the eventual correlation of immunostaining status with clinical outcome (correlation of positive immunostaining with the development of lung cancer and vice versa). Immunostaining for this study will be done at the University of Pennsylvania, and data acquisition and analysis will be handled at Johns Hopkins University. As part of this effort, selected patients will undergo bronchoscopy, and their bronchial lavage fluids will be studied for the quantity and quality of growth factor expression. We have developed a variety of methods for assessing the proliferative capacity of bronchial lavage products in an effort to complement the sputum immunocytology approach in determining who is and is not at risk for manifesting lung cancer.

Preliminary analysis of the characteristics of the first half of the patient accrual was recently published. A series of papers from this study summarizing the preliminary biochemical and molecular analysis of the bronchial lavages are being finalized.

Core analysis includes quantitation of autocrine growth factors such as GRP as well as more global assessment analysis of neuroendocrine activation by monitoring levels of peptidyl amidating monooxygenase (PAM) activity. This application builds upon the biology elucidated in our lab, establishing the role of this enzyme system in contributing to the chronic growth stimulation of neoplastic pulmonary epithelium. This work has major relevance in developing new early lung cancer detection approaches.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (60 characters or less. Title must fit on one line between the borders.)

Identification of Peptide Growth Factors That Regulate Human Tumor Proliferation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	F. Cuttitta	Deputy Branch Chief	BPRB, EDCOP, DCPC, NCI
Others:	K. Quinn	Post-Doctoral Fellow	BPRB, EDCOP, DCPC, NCI
	E. Unsworth	Chemist	BPRB, EDCOP, DCPC, NCI
	M. Miller	Biologist	BPRB, EDCOP, DCPC, NCI
	A. Martinez	Visiting Scientist	BPRB, EDCOP, DCPC, NCI

COOPERATING UNITS (if any)

University of Pittsburgh, Pittsburgh, PA (J. Siegfried)

LAB/BRANCH

Biomarkers and Prevention Research Branch, EDCOP, DCPC

SECTION

Experimental Intervention Section

INSTITUTE AND LOCATION

National Cancer Institute, Rockville, Maryland

TOTAL STAFF YEARS:

2.4

PROFESSIONAL:

1.9

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human Subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Iron plays a critical role in the proliferation of all eukaryotic cell systems by functioning as a co-factor for the enzyme ribonucleotide reductase which converts the ribo sugar to its deoxy derivative (essential sugar backbone of DNA) and is an integral element of the electron transport mechanism involved in energy production (ATP generation). Most mammalian cell satisfy their iron requirement by selective uptake of this element from the external environment via transferrin (Tf)/transferrin receptor (TfR) interaction. Tf is a serum protein (80,000 daltons), capable of binding two molecules of iron. When iron loaded, the protein binds to high affinity receptors on the cell surface (TfR), ligand/receptor complex is internalized, iron is released intercellularly, and Tf/TfR complex is recycled back to the cell surface and Tf released. The liver is the primary organ responsible for Tf production, although several other tissue sites have been found to produce this protein including brain, lung, and testis. It has been previously shown that all tumor cells express high levels of TfR consistent with their high mitotic index. In addition, based on our earlier work with tumor cell lines grown in protein-free media (R0), we have demonstrated that acclimated cells could produce a Tf-like protein. We have therefore undertaken an extensive investigative study to evaluate if authentic Tf can be produced in R0 tumor cells lines and whether this protein is a crucial component in the carcinogenesis process of malignant disease. To accomplish this task we used both molecular probes and biochemical analysis to characterize the Tf-like entity produced by R0 tumor cells and pathological specimens. In 35 lines of different R0 tumor cell type (lung, colon, breast, ovarian, prostate, and neuroblastoma), the Tf and TfR mRNA have been co-identified by RT-PCR. These cells (lysates and conditioned media) were shown to produce an 80 kDa protein which expressed common immunological epitopes of human Tf. In several cell lines the product was further studied by amino acid sequence analysis and shown to be authentic Tf. When 22 pathological specimens of human lung carcinomas (small cell, adeno, squamous cell, bronchioloalveolar, and large cell) were examined by in situ PCR and immunohistochemistry, 80% of these tumors expressed Tf. In addition, normal adult human lung was shown to selectively express Tf mRNA in distinct pulmonary cell types; these include the ciliated columnar cells of the bronchus, certain cells of the serous gland, certain capillary epithelium cells and intermittent pockets of inflammatory cells within the stromal area of the bronchus. Our research findings constitute the first collective study implicating Tf/TfR as a possible autocrine mechanism underlying malignant disease.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Post-Translational Processing Mechanisms in Tumor Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: A. M. Treston Visiting Scientist BPRB, EDCOP, DCPC, NCI
Others: A. Martinez Visiting Scientist BPRB, EDCOP, DCPC, NCI
M. Foo Biologist BPRB, EDCOP, DCPC, NCI
S. Ghosh summer student
N. Goldberg summer volunteer

COOPERATING UNITS (if any)

LAB/BRANCH

Biomarkers and Prevention Research Branch, EDCOP, DCPC

SECTION

Experimental Intervention Section

INSTITUTE AND LOCATION

National Cancer Institute, Rockville, Maryland

TOTAL STAFF YEARS:

3.5

PROFESSIONAL:

2.25

OTHER:

1.25

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human Subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Amidated peptide hormones are an important class of tumor growth factors in endocrine lung tumor cells and are potentially important in the regulation of tumor progression in lung and other tumor types. Our studies of tumor cell enzymes required for processing of precursor prohormones to active peptide hormones are comprised of three parts: the biochemistry of peptidyl amidating enzymes (PAM), the biology of PAM in human tumors, and the effect on cell growth of inhibiting PAM.

The two enzymes responsible for peptide amidation (PHM and PAL) are synthesized from the same gene and mRNA precursor, and we have confirmed that in human tumor cell lines they function both as two separate enzyme activities and as a linked bifunctional enzyme. We developed pcr-based techniques to enable us to differentiate the forms of these enzymes at the mRNA level. We have identified several novel forms of the linker region between the PHM and PAL domains of PAM which involve an exon specific to humans. Alternative mRNA splicing at the transmembrane domain appears to be similar to that reported in other species. We are confirming the presence of these new forms and characterizing the translated enzymes biochemically.

We previously reported that non-neuroendocrine non-small cell lung cancer cell lines had unexpectedly high levels of PAM enzymes. This led us to explore PAM expression in several tumor types generally considered to be non-neuroendocrine. We have found high levels of expression of PAM in ovarian tumor cell lines but low expression in colon, prostate, and breast lines. We are expanding these studies via an approved clinical protocol to obtain tissue samples for these tumors to study PAM expression in tumors directly using immunochemical methods and in tissue extracts with our biochemical assay.

Several of the small cell lung cancer cell lines which express PAM have been reported to be dependent on self-stimulatory growth loops involving amidated peptides. We previously used growth assays to explore whether inhibition of PAM would result in growth inhibition of these cell lines. Two classes of compounds did inhibit tumor cell growth. To confirm that this inhibition is specific to PAM, we are repeating these studies using transfected cell lines expressing antisense PAM mRNA. The data from these experiments support our original findings. We are continuing these studies using chemical inhibitors in a nude mouse xenograft model.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biologic Specimen Bank for Early Lung Cancer Markers in Chinese Tin Miners

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: P. R. Taylor Branch Chief CPSB, DCPC, NCI

Others: M. Forman	Nutritional Epidemiologist	CPSB, DCPC, NCI
Y. L. Qiao	Visiting Associate	CPSB, DCPC, NCI
M. M. Maher	Research Study Coordinator	CPSB, DCPC, NCI
S. B. Green	Lead Research investigator	CDTS, BB, DCPC, NCI

COOPERATING UNITS (if any)

Yunnan Tin Corporation
Johns Hopkins University School of Hygiene and Public Health
Division of Cancer Etiology, NCI
Biometry Branch, DCPC, NCI

LAB/BRANCH

Cancer Prevention Studies Branch, CPRP, DCPC

SECTION

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.0

PROFESSIONAL:

1.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human Subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Lung cancer is the leading cause of death from malignant neoplasms in the United States. Reduction in the mortality from this lethal malignancy will require reduction in the prevalence of risk factors and/or improved diagnosis and therapy. While relative survival rates for localized disease are dramatically better than for nonlocalized disease, most patients are not diagnosed early enough for present therapies to be effective. Advances in our understanding of the biology of lung cancer in recent years indicate that research to identify early markers of lung cancer may hold great promise for the reduction of lung cancer mortality. Numerous potential candidates for the early detection of lung cancer in sputum exist.

The tin miners at the Yunnan Tin Corporation (YTC) in China have an extremely high rate of lung cancer. Among high risk miners, defined as 40+ years old with 10+ years of underground mining and/or smelting experience, lung cancer rates exceed 1% per year. These extraordinary lung cancer rates result from combined exposure to radon, arsenic, and tobacco smoking in the form of cigarettes and/or bamboo water pipe.

The primary objective of this study is to establish a biologic specimen bank and data bank that can be used for the validation and refinement of potential early markers of lung cancer. Biologic specimens to be collected include sputum, blood, urine, and toenails. A secondary objective includes the establishment of a cohort for the study of environmental (including dietary) and genetic risk factors for lung cancer.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Research in Statistical Methodology and Consultation for Cancer Prevention

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PIs:	L. S. Freedman	Acting Branch Chief	BB, DCPC, NCI
	D. L. Levin	Senior Research Investigator	BB, DCPC, NCI
	V. Kipnis	Visiting Scientist	BMCCES, BB, DCPC
	B. Graubard	Mathematical Statistician	CDTS, BB, DCPC, NCI
Others:	A. M. Hartman	Health Statistician	ARB, DCPC, NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Biometry Branch, OD, DCPC

SECTION

Office of the Chief

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.0

PROFESSIONAL:

1.8

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human Subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to conduct statistical research and provide consultation to the Division for clinical trials, laboratory experiments, and epidemiological studies relevant to cancer prevention and control.

Research problems under investigation include statistical models for monitoring prevention trials; design of nutritional cohort studies including two-stage designs that allow construction of a followup cohort with a greater variation of nutrient intake; design of studies to calibrate dietary assessment instruments, allowing repeated assessments of two different instruments, one of which is unbiased; use of Bayesian methods for monitoring clinical trials; studies on a large observational database of HIV-infected subjects with evaluation of natural history of disease; and the design of trials for the prevention of recurrence of polyps.

Statistical consultation is provided to numerous studies including the NIH Women's Health Initiative Clinical Trial and the Polyp Prevention Trial. The consultation has involved extensive contributions to study design, study analysis and in the case of the Polyp Prevention Trial, continual advice on the day-to-day operations and on data monitoring.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Transcription Factors in Breast Epithelial Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	P. H. Brown	Senior Investigator	BPRB, EDCOP, DCPC, NCI
Others:	M. J. Birrer	Section Chief	BPRB, EDCOP, DCPC, NCI
	L. M. Smith	IRTA Fellow	BPRB, EDCOP, DCPC, NCI
	T. K. Chen	Biologist	BPRB, EDCOP, DCPC, NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Biomarkers and Prevention Research Branch, EDCOP, DCPC

SECTION

Molecular Mechanisms Section

INSTITUTE AND LOCATION

National Cancer Institute, Rockville, Maryland

TOTAL STAFF YEARS:

3.0

PROFESSIONAL:

3.0

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human Subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this study is to define the activity of nuclear transcription factors involved in regulating the proliferation and transformation of human breast epithelial cells, with the long term goal of identifying new targets for future chemopreventive agents. Such transcription factors include those activated by peptide hormones, such as epidermal growth factor (EGF), insulin-like growth factors (IGFs), and the heregulins, as well as those activated by the steroid hormones, such as estrogen, progesterone, and retinoic acid. We are presently studying the transcription factors in breast epithelial cells which are activated by mitogenic peptide hormones, such as the Jun/Fos, STAT, Myc/Max, and C/EBP families of transcription factors.

Over the last year, we have characterized Jun and Fos transcription factor activity in normal human mammary epithelial cells, in non-tumorigenic, immortal human breast epithelial cells, and in breast cancer cells. Multiple growth factors, including those important for controlling the growth of breast epithelial cells, such as EGF and IGF-1, induce activation of the Jun and Fos transcription factors in these cells. In addition, an inhibitor of Jun and Fos blocks this growth factor-induced activation of these transcription factors. We have also studied the expression and activity of the STAT and myc/max transcription factors, and have shown that these transcription factors are expressed in human breast cancer cells. A detailed characterization of their expression and activity in human mammary cells will allow us to determine the relative role of each of these transcription factor families in controlling breast cell proliferation and transformation. We are now attempting to use specific transcription factor inhibitors to block growth factor-induced proliferation and oncogene-induced transformation of human breast epithelial cells. By interfering with transcription factor function, we may be able to block signal transduction pathways at a distal point where the signals from multiple growth factors converge, and prevent proliferation or transformation of breast epithelial cells. Thus, these studies may identify new cellular targets for future chemopreventive agents.

PERIOD COVERED

June 1, 1993 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Evaluation of Markers for the Early Detection of Breast Cancer

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	G. L. Shaw	Senior Investigator	BPRB, EDCOP, DCPC, NCI
Others:	P. Brown	Senior Investigator	BPRB, EDCOP, DCPC, NCI
	J. L. Mulshine	Chief	BPRB, EDCOP, DCPC, NCI
	E. Szabo	Senior Investigator	BPRB, EDCOP, DCPC, NCI
	S. Jakolew	Senior Investigator	BPRB, EDCOP, DCPC, NCI
	I. Avis	Biologist	BPRB, EDCOP, DCPC, NCI

COOPERATING UNITS (if any)

GlycoTech, Rockville, MD (J. L. Magnani)

LAB/BRANCH

Biomarkers and Prevention Research Branch, EDCOP, DCPC

SECTION

Experimental Intervention Section

INSTITUTE AND LOCATION

National Cancer Institute, Rockville, Maryland

TOTAL STAFF YEARS:

1.75

PROFESSIONAL:

1.25

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human Subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

While mammography provides a method for the early detection of breast cancer, there are still breast cancer patients who do not have mammographically detectable lesions. Furthermore, only 25% of women who develop breast cancer have a recognized risk factor. Evaluation of the ductal epithelium of the breast may reveal markers which could identify women who are at an increased risk for developing breast cancer and thereby would derive greater benefit from surveillance or would be appropriate for intervention studies. Breast ductal epithelium is shed into ductal fluid, and this fluid can be aspirated from the nipple in approximately 50-60% of women. Published studies by Petrakis and others have shown that the ability to yield fluid is associated with an increased risk of breast cancer compared to non-yielders, but proliferative cytology alone is not adequate to predict breast cancer occurrence.

A protocol will be submitted for a feasibility trial to determine the acceptability of obtaining breast nipple aspirate fluid, to characterize the range of volumes obtained and to develop methods of performing multiple assays of the fluid obtained. Specimens will be examined for expression of markers including IGF-1 or TGF- β levels, estrogen and progesterone receptors, retinoic acid receptors, erbB-2 expression, carbohydrate antigen expression and others. Breast duct aspirate and breast needle aspirates would be obtained to characterize marker expression detectable in one or both specimen sources for concordance. An intervention trial with tamoxifen is planned in a very high risk population identified by traditional risk factors to determine whether marker expression changes with tamoxifen administration. Serial specimens would be examined for modulation of marker expression in response to the intervention. Pharmacologic investigations are proposed to examine the lowest dose of these agents which are associated with a biologic effect. Women with abnormalities on screening mammography would be stratified by whether biopsy of the abnormality was recommended based on standard clinical practice. The characterization of the association between mammographic findings and biomarker expression should be a useful adjunct to the management of these women.

Collaborations with the NIH Nuclear Medicine Department are proceeding to develop a PET mammography unit for structural correlations of biochemical changes in the breast detected by PET scanning. These changes will be evaluated in high risk women and correlated with marker findings.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Molecular Genetics of Gynecologic Cancers

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M. J. Birrer Senior Investigator BPRB, EDCOP, DCPC, NCI

Others: S. Lemon Cancer Prevention Fellow BPRB, EDCOP, DCPC, NCI

COOPERATING UNITS (if any)

Department of OB-GYN, Navy Medical Center (M. Parker, R. Taylor, J. Nash)
Armed Forces Institute of Pathology (J. Norris)

LAB/BRANCH

Biomarkers and Prevention Research Branch, EDCOP, DCPC

SECTION

Molecular Mechanisms Section

INSTITUTE AND LOCATION

National Cancer Institute, Rockville, Maryland

TOTAL STAFF YEARS:

2.0

PROFESSIONAL:

2.0

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human Subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Gynecologic cancers remains a major health problem for women in this country with approximately 25,000 deaths annually attributed to these cancers. The purpose of this project is to characterize the molecular genetics of this group of tumors and ultimately use that information for clinical application in designing therapeutic and prevention trials.

We have characterized 63 ovarian tumors which span the histologic spectrum from benign cystadenomas through tumors of "low malignant potential" to ovarian carcinomas for mutations in the *ras*, P53, and Rb oncogenes. Results from this study revealed that activated *ras* genes are found in 10% of benign cystadenomas and 30% of "LMP" tumors but not in ovarian carcinomas. In addition, mutations in the tumor suppressor genes P53 and Rb occur in ovarian carcinomas (48 and 14% respectively) but are not present in LMP tumors. This suggested that these tumors are discrete biologic entities. Of further interest, the incidence of activated *ras* genes in LMP tumors is higher in more advanced tumors suggesting it is a negative prognostic factor for these tumors. Future projects will examine the value of p53 mutations as a prognostic or an early detection marker in ovarian cancers by examining large numbers of early and advanced stage ovarian cancers.

We are also examining endometrial specimens which span the histologic spectrum from benign to malignant for mutations in the *ras* and p53 genes. Activated *ras* genes are found in the atypical hyperplasias (14%) and endometrial carcinomas (5%). P53 mutations rare found in approximately 15% of hyperplasia and carcinomas. These studies will help to identify the molecular genetic events which are important in the genesis of these tumors. In addition, it will characterize the temporal relationship among these events enabling one to determine if any of these molecular lesions can be used as markers of early disease.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemistry of Peptides and Growth Factors in Lung Cancer

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: T. W. Moody Section Chief
Others: A. Mathias TechnicianBPRB, EDCOP, DCPC, NCI
BPRB, EDCOP, DCPC, NCI

COOPERATING UNITS (if any)

University of Arizona College of Medicine (T. P. Davis)
Tel Aviv University (I. Gozes)

LAB/BRANCH

Biomarkers and Prevention Research Branch, EDCOP, DCPC

SECTION

Experimental Biochemistry Section

INSTITUTE AND LOCATION

National Cancer Institute, Rockville, Maryland

TOTAL STAFF YEARS:

1.7

PROFESSIONAL:

1.5

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human Subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The biochemistry of peptides and growth factors in lung cancer was investigated. Bombesin/gastrin releasing peptide (BB/GRP) is a positive autocrine growth factor for small cell lung cancer (SCLC) and the growth of SCLC is inhibited by synthetic receptor antagonists. In our studies, GRP gene expression was increased by phorbol-12-myristate-13 acetate (PMA), which activates protein kinase C (PKC). The increase in GRP mRNA caused by PMA was reversed by (5-isoquinolinesulfonyl)-2-methyl-piperazine (H7). We also found that PMA increased SCLC growth and H7 significantly inhibited proliferation. These data suggest that PKC may be an important enzyme mediating SCLC growth. Also, SCLC growth was inhibited by the chemopreventive protease inhibitor Bowman-Birk inhibitor (BBI). BBI decreased levels of bioactive GRP levels but increased levels of inactive proGRP. BBI may inhibit enzymes which process GRP.

Transforming growth factor α /epidermal growth factor (TGF α /EGF) is a positive autocrine growth factor for non-SCLC (NSCLC). Here a TGF α -pseudomonas exotoxin (TGF α -PE40) chimera bound with high affinity to the EGF receptor. TGF α -PE40 was internalized by NSCLC cells and the PE40 metabolites were released into the cytosol inhibiting protein synthesis. As a result, TGF α -PE40 killed NSCLC cells *in vitro* and *in vivo*. TGF α -PE40 was cytotoxic for NSCLC cells. In contrast, thymosin α 1 (THN α 1) was cytostatic for NSCLC cells. THN α 1 inhibited colony formation using cell biology techniques and xenograft formation in nude mice in a reversible manner. THN α 1 also stimulated arachidonic acid (AA) release from NSCLC cells. THN α 1 may activate phospholipase A₂ stimulating AA releases from endogenous phospholipids. These data suggest that THN α 1 may be a negative autocrine growth factor for NSCLC cells.

PERIOD COVERED

June 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Evaluation of Markers for the Early Detection of Lung Cancer

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	G. L. Shaw	Senior Investigator	BPRB, EDCOP, DCPC, NCI
Others:	J. L. Mulshine	Chief	BPRB, EDCOP, DCPC, NCI
	F. Cuttitta	Deputy Chief	BPRB, EDCOP, DCPC, NCI
	S. Jakolew	Senior Investigator	BPRB, EDCOP, DCPC, NCI
	E. Szabo	Senior Investigator	BPRB, EDCOP, DCPC, NCI
	H. Pass	Chief	TOS, SB, COP, DCT, NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Biomarkers and Prevention Research Branch, EDCOP, DCPC

SECTION

INSTITUTE AND LOCATION

National Cancer Institute, Rockville, Maryland

TOTAL STAFF YEARS:

1.75

PROFESSIONAL:

1.25

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human Subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The early detection of lung cancer is critical to improving the mortality rate associated with lung cancer. Protocols for the early detection of lung cancer among individuals at high risk will be submitted for approval. We propose to target lung cancer and head and neck cancer survivors with serial monitoring of sputum and bronchoscopically obtained specimens to assay for biomarker expression. The study design will incorporate comparison of findings in different specimens such as bronchial washings, bronchial biopsies at multiple sites and expectorated sputum. Particularly important would be whether or not the markers are differentially expressed in the unaffected portions of the lung or only detectable in certain types of specimens.

A subset of subjects will be enrolled in an intervention trial. Specimens will be obtained at on-study, after the period of intervention and roughly 3 months after the intervention is stopped. The first intervention trial anticipated will use 4-HPR if available, or low dose 13-*cis*-retinoic acid. Future intervention agents for this subject population might include Vitamin E or the radiolabeled photoactive porphyrin compound for a 3-6 month period. Pharmacokinetic studies will also be incorporated to evaluate the effect of dose on marker modulation as well as to evaluate alternative forms of administration such as aerosolization of the agents. The success of early detection of cancer is dependent upon the ability to intervene successfully at that early stage to prevent the morbidity and mortality associated with cancer and standard therapy.

Published reports have suggested that genomic p53 mutations may be present in individuals with an inherited susceptibility to several types of cancer, including but not limited to Li-Fraumeni syndrome. A similar study using genomic DNA from whole blood collected during a case-control study of lung cancer is planned to evaluate markers of susceptibility to lung cancer.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Prediagnostic Breast Cancer Serum Bank (Columbia, Missouri)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)

PI: J. Dorgan Senior Staff Fellow CPSB, DCPC, NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Cancer Prevention Studies Branch, CPRP, DCPC

SECTION

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.20

PROFESSIONAL:

0.15

OTHER:

0.05

CHECK APPROPRIATE BOX(ES)

☒ (a) Human Subjects☒ (b) Human tissues☐ (c) Neither☐ (a1) Minors☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A major obstacle to our understanding of the etiology of breast cancer is the lack of studies with prediagnostic serum for analysis. This year, the Cancer Prevention Studies Branch assumed responsibility for the Columbia, Missouri collection of the Breast Cancer Serum Bank from the Division of Cancer Biology, Diagnosis, and Centers, NCI. This collection includes serum collected between 1977 and 1987 from 7,641 women, 112 of whom subsequently developed breast cancer. Questionnaires on reproductive history, use of exogenous estrogens and other medications, and cancer diagnoses and treatment were completed at each blood drawing.

We currently are evaluating estrogen and androgen levels in serum from 74 women who developed postmenopausal breast cancer and 148 matched controls. A study to evaluate pesticide and PCB levels in serum from cases and noncases is being developed and a study to analyze antioxidants is planned.

Analyses of serum from the Breast Cancer Serum Bank will improve our understanding of the role of diet and hormones, as well as environmental exposures, in the etiology of breast cancer.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Early Detection of Esophageal Cancer

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: S Dawsey Senior Staff Fellow CPSB, CPRP, DCPC, NCI
Others: P Taylor Branch Chief CPSB, CPRP, DCPC, NCI
D. Solomon Section Chief LP, DCBDC, NCI

COOPERATING UNITS (if any)

Cancer Hospital, Chinese Academy of Medical Sciences
Georgetown University
University of California at Los Angeles

LAB/BRANCH

Cancer Prevention Studies Branch, CPRP, DCPC

SECTION

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.5

PROFESSIONAL:

0.5

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human Subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Esophageal cancer is a common malignancy with a very poor prognosis. The principal reason for the poor prognosis is that most tumors are asymptomatic and go undetected until they have spread beyond the esophageal wall and are unresectable. Significant reduction in esophageal cancer mortality will require successful strategies to diagnose a greater proportion of cases at earlier, more curable stages of disease. A successful early detection program for esophageal cancer will require an accurate, patient-acceptable screening test, confirmatory tests that can localize precursors and early invasive lesions, and a curative therapy that is acceptable to asymptomatic patients. This project includes four studies, each designed to evaluate a technique which may be useful in such an early detection program:

- The Cytology Sampling Study will estimate and compare the sensitivity of the two currently available cytological sampling techniques, the Chinese balloon and the Japanese encapsulated sponge, for identifying biopsy-proven squamous dysplasia and cancer of the esophagus.
- The Mucosal Staining Study will evaluate whether mucosal iodine staining can improve endoscopic localization of esophageal squamous dysplasia and cancer.
- The Endoscopic Ultrasonography Study will evaluate how accurately ultrasonography can identify and stage squamous dysplasia and early invasive cancer of the esophagus.
- The Endoscopic Therapy Pilot Study will evaluate the feasibility, safety, acceptability, and preliminary efficacy of mucosectomy, a focal endoscopy therapy which has recently been developed in Japan. Currently, standard treatment for early esophageal cancer is esophagectomy, a large operation that is not acceptable to many asymptomatic patients. For an early detection program to decrease esophageal cancer mortality, we must develop an alternative cure that is acceptable to such patients.

This project will be carried out in Linxian, China, a county with extraordinary rates of esophageal cancer and a correspondingly high prevalence of the asymptomatic precursor and early invasive lesions that are needed for these studies. The project will be a collaborative effort of investigators from the National Cancer Institute, the Cancer Hospital of the Chinese Academy of Medical Sciences, Georgetown University, and the University of California at Los Angeles.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

NCI-AARP Health Study

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: A Schatzkin, Medical Officer, CPSB, DCPC, NCI

Others:	L. S. Freedman	Acting Branch Chief	BB, DCPC, NCI
	C. C. Brown	Section Chief	BB, DCPC, NCI
	A. Subar	Nutritionist	ARB, DCPC, NCI
	F. Thompson	Epidemiologist	ARB, DCPC, NCI
	J. A. Tangrea	Deputy Branch Chief	CPSB, DCPC, NCI
	N. Potischman	Senior Staff Fellow	EEB, DCE, NCI

COOPERATING UNITS (if any)

Applied Research Branch and Biometry Branch, DCPC; Environmental Epidemiology Branch, DCE; American Association of Retired Persons

LAB/BRANCH

Cancer Prevention Studies Branch, CPRP, DCPC

SECTION

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.5

PROFESSIONAL:

1.5

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human Subjects ☐ (b) Human tissues ☐ (c) Neither☐ (a1) Minors☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objective of this project is to carry out a prospective cohort study of diet in relation to several major cancers, especially breast, prostate and colorectal malignancies. The cohort will consist primarily of male and female members of the American Association of Retired Persons (AARP). In order to guarantee adequate heterogeneity of dietary intake for key nutrients and foods, the cohort will be established in a two-phase process. Food frequency questionnaires will initially be sent to 3.5 million AARP members. All respondents falling within the extreme categories of dietary intake (e.g., for fat, vegetables, or various 'dietary pattern' combinations of nutrients and foods) will be selected for the final cohort, along with a random sample of those falling within the intermediate categories of intake. The final cohort will consist of 350,000 persons, half men, half women. Oversampling of minority AARP members will be done to ensure an adequate representation of minorities in the final cohort. Validation/calibration studies of the food frequency questionnaire will be carried out within the first year of the contract period. A second questionnaire, comprising questions on exposures not assessed in the first questionnaire, will be mailed to cohort members several months after the initial questionnaire. A brief followup questionnaire, primarily targeted to endpoint assessment, will be mailed to cohort members at the end of the five year period of observation. Followup will be largely passive, through established state registries. The initial questionnaire mailing will be to AARP members in those states selected on the basis of having registries with adequate coverage and quality. Active followup with record retrieval will be conducted for the small percentage of cohort members who have moved out of the cancer registry areas.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Statistical Inference in Model Selection

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: V. Kipnis

Others: D. Midthune Information Management Services, Inc.
C. Heuer German Cancer Research Center

COOPERATING UNITS (if any)

Information Management Services, Inc.
German Cancer Research Center

LAB/BRANCH

Biometry Branch, OD, DCPC

SECTION

Biostatistical Methodology and Cancer Control Epidemiology Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

0.8

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human Subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The focus of this project is development and refinement of statistical procedures for evaluating and selecting regression models. Problems under investigation include two areas: multi-step variable selection in multiple regression analysis and discriminating among alternative model specifications, including non-nested classes of models.

Although various variable selection procedures have found widespread application in biostatistics and epidemiology, e.g., in the analysis of case-control and cohort studies with many risk factors, their statistical properties are quite poorly understood. In particular, statistical inference both at intermediate steps and for the finally chosen model is problematic. Many stepwise procedures are based on repeated tests of significance. Theory has been developed that addresses the problem of derivation of the distribution for the F-ratio at each step of a sequential forward selection. It is shown that beginning with the second step, the distribution of the F-ratio involves some nuisance parameters, but an appropriate conditioning leads to an exact conservative test. It is also shown that the conventional cut-off values based on the central F-distribution lead to a "liberal" test that does not control the Type I error. A FORTRAN program has been developed jointly with D. Midthune to calculate the correct cut-off values at each step of the procedure.

Many alternative model specifications in applied biostatistical studies contain non-nested classes. Methodology is being developed for discriminating among non-nested models. The approach is based on a nonparametric relevancy criterion that evaluates each model by comparing its performance for the observed data and generated pseudo-data with no relationship between response and explanatory variables. The computer simulations demonstrate that this criterion has better statistical properties than many known procedures. Current research is being conducted jointly with D. Midthune and C. Heuer and includes application of this approach to discriminating among different "age-period-cohort" log-linear models.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Statistical Inference in Stochastic Regression Models

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: V. Kipnis

COOPERATING UNITS (if any)

LAB/BRANCH

Biometry Branch, OD, DCPC

SECTION

Biostatistical Methodology and Cancer Control Epidemiology Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.6

PROFESSIONAL:

0.5

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human Subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is development of the statistical theory for possibly misspecified stochastic regression models and their applications in assessing the association between disease and explanatory variables. The current research is focused on linear stochastic regression and the effect of possible misspecification such as nonlinearity and heteroscedasticity. Theory is being developed that provides the asymptotic unconditional distribution of the least squares estimators of the regression coefficients in such models. In particular, it is shown that the expected values of the estimators are asymptotically the same as in conventional regression analysis, but the formulas for the standard errors of the estimated regression coefficients differ from those for the correctly specified models.

The theory is being used to derive some new results in two applied areas. Firstly, to investigate the consequences of discretizing continuous explanatory variables by categorizing them into a small number (3-5) of groups, e.g., quartiles, or ordering them so as to investigate trend over these groups, as is routinely done in epidemiologic studies. The second problem under investigation is to assess the effect of measurement errors in the explanatory variables. Theory has been developed to evaluate the general case when measurement errors correlate among themselves and with the true values of the covariates, as is often the case with self-reported variables such as nutritional intakes.

The results of this work are being applied to evaluate the statistical properties of three alternative energy-adjustment models in nutritional epidemiology.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Dietary Regulation of Biochemical/Molecular Change in Carcinogen Resistant Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	G.C.Yeh	Senior Investigator	LNMR,CPRP,DCPC,NCI
Others:	J.Lopaczynska	Visiting Fellow	LNMR,CPRP,DCPC,NCI
	J.M.Phang	Lab Chief	LNMR,CPRP,DCPC,NCI
	C.A.Plouzek	Senior Staff Fellow	LNMR,CPRP,DCPC,NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Nutritional and Molecular Regulation, CPRP,DCPC

SECTION

INSTITUTE AND LOCATION

NCI-Frederick Cancer Research and Development Center, Frederick, Maryland

TOTAL STAFF YEARS:

1.2

PROFESSIONAL:

1.2

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human Subjects
 ☐ (b) Human tissues
 ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We previously demonstrated that chemical carcinogens, benzo(a)pyrene and 7, 12-dimethylbenz-anthracene (DMBA) efflux mediated by the multidrug resistant (MDR) glycoprotein 170 (P-gp) in human breast cancer MCF-7 cells. We recently developed resistant human breast cancer cells resistant to benzo(a)pyrene (BP) by exposing them to increasing concentrations of this well known carcinogen. We found the BP resistant cells also were co-resistant to other carcinogen, e.g., DMBA but not to chemotherapeutic drugs, e.g. adriamycin, vinblastine. Importantly, this series of BP resistant MCF-7 cells neither express *mdr* RNA nor produced P-gp protein. From our findings we conclude that the mechanism for resisting the cytotoxicity of carcinogens is not due to P-gp-mediated carcinogen efflux. We carefully examined the biochemical and molecular changes in BP resistant cells and found the major changes are associated with repair mechanisms. A marked increase in enzyme activities and RNA expressions of glucose-6-phosphate dehydrogenase (G6PD), hypoxanthine-guanine phosphoribosyl transferase (HGPRT), and Topoisomerase II (Topo II) in BP resistant cells than carcinogen sensitive wild type cells.

The implications of the increase in a glucose metabolic enzyme (G6PD), purine salvage pathway enzyme (HGPRT) and DNA repair enzyme (Topo II) are being investigated. Of special interest will be the regulation of these enzymes by dietary factors. We hope the understanding of carcinogen resistance in tissue culture cells will enhance our knowledge of this important mechanism in cancer prevention.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanisms of Diet and Chemoprevention in p53-Knockout Transgenic Mice

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	S. Hursting	Cancer Prev. Fellow	LNMR, CPRP, DCPC, NCI
Others:	J. Phang	Chief	LNMR, CPRP, DCPC, NCI
	S. Perkins	Senior Staff Fellow	LNMR, CPRP, DCPC, NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Nutritional and Molecular Regulation, CPRP, DCPC

SECTION

INSTITUTE AND LOCATION

NCI-Frederick Cancer Research & Development Center, Frederick, Maryland

TOTAL STAFF YEARS:

1.4

PROFESSIONAL:

1.4

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human Subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Mice with the p53 tumor suppressor gene knocked out by gene targeting develop normally but have increased susceptibility to spontaneous tumor development. We are characterizing and using nullizygous p53-knockout mice as a model of genetic susceptibility to screen for nutritional and chemopreventive agents which can offset the increased risk of tumorigenesis resulting from the loss of tumor suppressor function. We have shown that calorie restriction (CR; a potent inhibitor of many types of rodent tumors) delays spontaneous tumor development in p53-knockout mice. We have also assessed the effect of the putative chemopreventive agents dehydroepiandrosterone (DHEA), quercetin, d-limonene and all-*trans* retinoic acid on spontaneous tumorigenesis in p53-knockout mice to further establish the efficacy of this model for testing cancer prevention strategies. DHEA, an inhibitor of glucose 6-phosphate dehydrogenase, impressively delays tumor development in p53-knockout mice, while the other agents have no significant effects. These studies established that p53-knockout mice provide a useful *in vivo* model of genetic susceptibility since tumorigenesis in these mice is spontaneous, rapid, relevant to human cancer and responsive to experimental manipulation.

Currently in progress are molecular studies on tissues collected serially from control, CR- and DHEA-treated wild-type and p53-knockout mice. We are analyzing tissues for differences in p53, WAF/p21, *ras*, *bcl-2*, *myc*, *cdc-2*, and *mdr* expression using Northern blot analysis and immunohistochemistry. We are also comparing differential messenger RNA expression in liver samples from these mice using the differential display reverse transcription-PCR technique. Other studies in progress include: 1) a pathological/immunological/virological characterization of tumors from p53-knockout mice; 2) an analysis of tumor development in p53-knockout mice in response to DHEA analogues to further characterize this class of chemopreventive compounds; 3) a 2-year study of CR effects on spontaneous tumorigenesis in wild-type mice which is providing evidence that CR modulates tumorigenesis through both p53-dependent and p53-independent mechanisms; 4) a study of CR effects on 2-stage skin carcinogenesis and inflammation/oxidant production in male hemizygous p53-knockout mice and 5) a comparison of the effects of different doses of chronic and acute nitrosomethylurea exposure in female hemizygous p53-knockout mice, testing the sensitivity of these mice to chemically-induced tumor development; 6) an assessment of the effects of CR on nitrosomethylurea-induced tumor development and mutation rates.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Control of P-glycoprotein Function by Iron

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	H. Ciolino	Staff Fellow	LNMR, CPRP, DCPC, NCI
Others:	G. C. Yeh	Senior Investigator	LNMR, CPRP, DCPC, NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Nutritional and Molecular Regulation, CPRP, DCPC

SECTION

INSTITUTE AND LOCATION

NCI-Frederick Cancer Research and Development Center, Frederick, Maryland

TOTAL STAFF YEARS:

1.2

PROFESSIONAL:

1.2

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human Subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The plasma membrane ATPase P-glycoprotein (P-gp) is believed to confer multidrug resistance (MDR) on cells by the active transport of chemotherapeutic drugs out of the cell. Also, it is present in normal tissues and may play a physiologic role in the protection of cells from dietary xenobiotics. Using adriamycin-resistant cells of the breast cancer cell line MCF-7 previously developed in this laboratory, we have investigated the regulation of P-gp by examining the accumulation and efflux of adriamycin and vinblastine. We have found that the activity of P-gp is affected by the level of intracellular iron. When the cells are loaded with iron using the lipophilic metal chelator 8-hydroxyquinoline or by culturing the cells in the presence of hemin, drug accumulation in the resistant cell lines increases to levels similar to that seen in the wild type cell. Drug efflux, normally quite rapid in the resistant cells, is abolished. Conversely, using metal chelators to lower the level of intracellular iron increases P-gp function. The regulation of P-gp by iron is a novel observation whose molecular mechanism is unknown. Since iron is involved in metal-catalyzed oxidation of macromolecules, including proteins, the possibility exists that the iron state of the cells affects P-gp through a redox-dependent mechanism. Oxidative modification of proteins causes several alterations in the amino acid side chains of susceptible residues, including the introduction of carbonyl groups. Oxidative inactivation of several enzymes has been demonstrated. The extent of oxidative modification in a protein or cellular protein extract may be assayed with the carbonyl reagent 2,4-dinitrophenylhydrazine. We propose to examine P-gp for oxidative modification using this assay. In order to discover whether MDR may be associated with oxidation, we further propose to investigate the relative susceptibility of the wild type and resistant MCF-7 cells to oxidative damage caused by iron, using the carbonyl assay and by examining the oxidative modification of DNA bases. A thorough understanding of the relationship between the redox state of the cell and the regulation of P-gp is important in determining the role of P-gp in the prevention of cancer.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Growth of Human Tumor Cell Lines in Protein-Free Media

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	F. Cuttitta	Deputy Branch Chief	BPRB, EDCOP, DCPC, NCI
Others:	K. Quinn	Post-Doctoral Fellow	BPRB, EDCOP, DCPC, NCI
	E. Unsworth	Chemist	BPRB, EDCOP, DCPC, NCI
	M. Miller	Biologist	BPRB, EDCOP, DCPC, NCI
	A. Martinez	Visiting Scientist	BPRB, EDCOP, DCPC, NCI

COOPERATING UNITS (if any)

University of Pittsburgh, Pittsburgh, PA (J. Siegfried)

LAB/BRANCH

Biomarkers and Prevention Research Branch, EDCOP, DCPC

SECTION

Experimental Intervention Section

INSTITUTE AND LOCATION

National Cancer Institute, Rockville, Maryland

TOTAL STAFF YEARS:

2.5

PROFESSIONAL:

2.0

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human Subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have previously demonstrated that most solid tumor cell lines of man have the ability to growth in protein-free media (R0). These include neoplasms of a diverse range of cell types encompassing lung, colon, breast, ovarian, pancreas, prostate, and brain. In contrast, none of the hemopoietic cell lines (lymphocytic and monocytic) we have studies were able to accomplish this R0 adaptation. We have noted that some cell lines undergo a morphological shift when adapted to R0. For example, the small cell lung cancer (SCLC) classic cell line NCI-H345, which grows as loose floating aggregates in serum supplemented media, will form tight floating spheroids under R0 conditions. The SCLC variant cell line NCI-N417 grows in loose floating aggregates in serum containing media also, but forms dendritic shaped adherent cell colonies in R0. Additionally, the adherent breast cancer cell line MCF-7 appears as a fibroblastoid shaped cell in serum but rapidly converts to a floating spheroid colony during R0 adaptation. The exact reason for the resulting morphological shift associated with growth in an R0 environment is not known but may possibly be a result of maximizing paracrine/autocrine growth factor availability.

Consistent with this idea, we have identified a variety of peptide/protein growth factors which are produced by R0 grown tumor cell lines; the most commonly found is insulin-like growth factor I (IGF-I) and transferrin (Tf). Utilizing an immune induction screening technique to assay the components of R0 conditioned media, we have identified immuno reactive species related to transforming growth factor α and β , epidermal growth factor, gastrin releasing peptide (GRP), insulin-like growth factor II, calcitonin, glucagon, nerve growth factor, fibroblast growth factor (acidic), arginine vasopressin, and vasoactive intestinal peptide. Interestingly, the production of such factors appears to be an inducible phenomenon which directly relates to the cell's ability to survive harsh nutritional surroundings. For example, we have demonstrated that the bronchioloalveolar carcinoma cell line A549, which normally does not produce GRP in serum supplemented media, will express GRP mRNA and secrete a functional growth-promoting peptide when adapted to R0 media (Siegfried *et al.*, JBC 269:8596-8603, 1994). In parallel studies, we have also shown that the message for IGF-I and Tf are upregulated during R0 adaptation. When concomitantly produced, both of these protein factors are potent inducers of cell proliferation which can mediate autocrine/paracrine growth of the tumor cell. By characterizing the cellular products responsible for R0 growth, we have begun to define the possible factors involved in the clonal expansion of tumor cells during carcinogenesis and reveal potential target sites for the disruption of this malignant process.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of Molecular Markers for Early Detection of Epithelial Tumors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	A.L. Sabichi	Research Associate	BPRB, EDCOP, DCPC, NCI
Others:	M.J. Birrer	Section Chief	BPRB, EDCOP, DCPC, NCI
	S.J. Lemon	Clinical Associate	BPRB, EDCOP, DCPC, NCI
	G.L. Shaw	Investigator	BPRB, EDCOP, DCPC, NCI
	M. Schiffman	Physician Epidemiologist	EES, EEB, EBP, DCE, NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Biomarkers and Prevention Research Branch, EDCOP, DCPC

SECTION

Molecular Mechanisms Section

INSTITUTE AND LOCATION

National Cancer Institute, Rockville, Maryland

TOTAL STAFF YEARS:

2.0

PROFESSIONAL:

1.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human Subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

The goals of this study are to use molecular biological techniques to identify and characterize the genetic aberrations in both preneoplastic lesions and tumors of epithelial origin, and to explore the potential use of these specific genetic lesions as markers for the early detection of cancer. Molecular alterations such as activation of oncogenes and loss of tumor suppressor genes have been shown to be important in early stages of the pathogenesis of many human neoplasms. We have chosen to study colorectal and endometrial cancers in which activating mutations of the *ras* oncogene and deletion or mutation of the tumor suppressor gene *p53* are frequently present.

Currently we are studying specimens obtained from 15 colorectal cancer patients accrued on a multicenter case control study carried out at the National Naval Medical Center, Bethesda, Maryland. Paired specimens obtained from each patient include a surgically excised colonic adenocarcinoma and preoperatively obtained and lyophilized stool specimens which contain shed epithelial cells. These specimens will be analyzed by PCR amplification, RFLP analysis, and sequencing for activated *Ki-ras* and *p53* mutations. Molecular analysis will also be performed on control subjects to assess the specificity of these tests. Subsequent statistical analysis will be done to identify the power of association between an identified abnormality in the DNA recovered from the primary tissue and stool samples.

We are also interested in identifying the molecular changes occurring in both premalignant lesions of the endometrium and endometrial cancers, and are presently characterizing the presence of *Ki-ras* and *p53* gene mutations in a group of endometrial curettage specimens spanning the histologic spectrum from benign to malignant. Our preliminary data show the presence of *Ki-ras* mutations in 6% of proliferative endometrium, 5% of simple hyperplasias, 14% of atypical hyperplasias, and 9% of carcinoma samples. Mutations in the *p53* gene were found in 6%, 5%, 7%, and 13% of proliferative endometrium, simple hyperplasia, atypical hyperplasia, and endometrial carcinoma specimens respectively. These results indicate that not only are these mutations likely to be initiating events in the oncogenesis of endometrial cancers, but that it is possible to detect these mutations in DNA extracted from small amounts of tissue to accurately assess the presence of early events in the development of neoplasms. We plan to look for other molecular aberrations which occur early in oncogenesis, might be present with higher frequency in endometrial lesions, and thus could be reasonably applied toward development of screening tests.

Furthermore, molecular analyses such as those described above will also be applied to other epithelial tumors with the intent of developing new screening modalities for the early detection of common epithelial malignancies.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Differentiation During Lung Carcinogenesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	E. Szabo	Investigator	BPRB, EDCOP, DCPC, NCI
Others:	R. I. Linnoila	Senior Investigator	BPRB, EDCOP, DCPC, NCI
	M. J. Birrer	Senior Investigator	BPRB, EDCOP, DCPC, NCI
	C. Corbett	Biologist	BPRB, EDCOP, DCPC, NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Biomarkers and Prevention Research Branch, EDCOP, DCPC

SECTION

Experimental Pathology Section

INSTITUTE AND LOCATION

National Cancer Institute, Rockville, Maryland

TOTAL STAFF YEARS:

1.3

PROFESSIONAL:

1.0

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human Subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The ability to redirect cellular processes from proliferation to terminal differentiation offers a promising approach toward preventing cancer progression. Over the past year we have begun to study differentiation in non-small cell lung cancer cell lines by three different approaches:

1) Evaluating the function and regulation of known differentiation antigens such as the Clara cell protein CC10. Analysis of CC10 mRNA expression reveals that the tumor promoter TPA and serum stimulation induce expression of CC10 in the H1334 cell line but not in H1299, both of which express CC10 by in situ hybridization. This is in spite of similarly high basal levels of *jun* and *fos* oncogenes (mediators of the TPA signal) and similar patterns of induction of these oncogenes by TPA and serum. Further studies using other biologically relevant agents and analysis using a human CC10 promoter-CAT construct are underway to clarify this and to determine the significance of altered CC10 expression to progressive pulmonary neoplasia.

2) Developing model systems of differentiation to enable the study of molecular mechanisms of differentiation control in lung carcinogenesis. We have previously demonstrated that TPA induced macrophage differentiation correlates strongly with *c-jun* expression. Our data in lung cancer shows that TPA does not have a similar effect (no growth cessation), perhaps because the induction of *jun/fos* is much lesser in magnitude and of shorter duration. We are currently also evaluating other potential differentiating agents such as the Na⁺/K⁺ ATPase inhibitor bufalin, signal transduction pathway inhibitors, and cytokines.

3) Using immunohistochemistry and flow cytometry to study differentiation markers and genes controlling differentiation and proliferation in primary lung tumors and premalignant lesions to identify targets for early detection screening. We have developed a technique for *jun* immunostaining and preliminary data shows that *jun* is highly expressed in primary lung tumors as well as in 5/6 cell lines. This will be correlated with *fos*, "downstream" genes (CD44, vimentin), prognosis in advanced cancer, and expression in premalignant lesions.

These studies will shed light on the biology of lung cancer and enable us to identify targets for early detection and intervention as well as potentially develop strategies for enforcing terminal differentiation as a therapeutic goal during lung carcinogenesis.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Consultation in Biostatistical Methodology and Cancer Control

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PIs: C. Brown Section Chief BMCCE, BB, DCPC, NCI
B. Patterson Mathematical Statistician BMCCE, BB, DCPC, NCI
Others: L. Freedman Acting Branch Chief OC, BB, DCPC, NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Biometry Branch, OD, DCPC

SECTION

Biostatistical Methodology and Cancer Control Epidemiology Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.1

PROFESSIONAL:

0.9

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human Subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to provide independent consultation to researchers within the NCI on problems related to biostatistical methodology and cancer control. Current projects include:

- in collaboration with the Cancer Prevention Studies Branch, design of the American Association of Retired Persons (AARP) Observational Cohort Study is continuing; this study is being designed to investigate the relationship between dietary intake and cancer of the breast, colon/rectum, and prostate; the contract was recently awarded, and the study will commence in the fall of 1994.
- in consultation with the Prevention and Control Extramural Research Branch, DCPC, statistical advice is being given to the Worksite Health Promotion Intervention Study; initial guidance was given on sample size calculations and pair matching of worksites and recent consultations have been concerned with statistical methods for the final analyses.
- in collaboration with the Cancer Prevention Studies Branch, DCPC, statistical analyses have been conducted on the effects of dietary energy, fat, and fiber intake on plasma levels of estrogens and androgens in 81 premenopausal women; dietary intake was measured by a food frequency questionnaire (FFQ) and a 7-day diet record; in addition, analyses have been also made on the effects of alcohol ingestion on plasma levels of estrogens and androgens in 107 premenopausal women.
- in consultation with the Applied Research Branch, DCPC, development of methods to evaluate the accuracy of the Cancer Information System services to the general public is continuing;
- in consultation with the DCPC Black-White Survival Study Group, a comparison is being made of the treatment patterns among black and white patients with in situ or early stage breast cancer.
- extensive statistical consultation with the 5-A-Day Program has continued; data from a baseline survey were analyzed and three manuscripts accepted for publication.
- in collaboration with the Epidemiology and Biostatistics Program, DCE, an analysis has been made of residential radon exposure in a case-control study of lung cancer among nonsmoking women.
- in collaboration with the Laboratory of Chemoprevention, DCE, a statistical analysis was made of the results from three animal studies examining the anticarcinogenic effect of adding either a retinoid or a newly developed synthetic vitamin D to Tamoxifen to the diets of rats exposed to MNU, a mammary carcinogen.
- in collaboration with the Environmental Epidemiology Branch, DCE, the effects of long-term storage and multiple freeze-thaw cycles on measurement of serum ascorbic acid were assessed using samples from a cervical cancer case-control study.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Community Intervention Trial for Smoking Cessation (COMMIT)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: S.B. Green Lead Research Investigator CDTS, BB, DCPC, NCI
D. K. Corle Computer Systems Analyst CDTS, BB, DCPC, NCI

COOPERATING UNITS (if any)

Information Management Services, Inc.

LAB/BRANCH

Biometry Branch, OD, DCPC

SECTION

Clinical and Diagnostic Trials Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.9

PROFESSIONAL:

0.8

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human Subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

This project provides consultation concerning statistical issues for COMMIT, a large-scale community-based study intended to promote smoking cessation among heavy smokers. Biometry Branch staff initially devised the basic design for the study—eleven matched pairs of communities with one member of each pair chosen at random for intervention and the other serving as a control. The study was designed to detect a 10% difference in the smoking quit rate between the intervention and control communities. Staff of the CDT Section have been actively involved in all meetings of the Steering Committee and have analyzed and presented data to the independent Policy Advisory Committee.

The COMMIT intervention ended in 1992, and the final surveys were performed in 1993, finishing in January 1994. The four principal final surveys were:

- 1) Endpoint Cohort Survey—The full cohorts of heavy and light-to-moderate smokers were contacted in the spring of 1993 to determine the self-reported, 6-month cigarette smoking cessation rates in each of the 22 communities. This measure is the primary endpoint of the COMMIT trial.
- 2) Cotinine Validation Study—After the completion of the Endpoint Cohort Survey, the "quitters" in the heavy smoker cohort were re-contacted for participation in this study. Saliva samples were collected from eligible and consenting participants for cotinine measurement. The study is designed to measure cessation misrepresentation rates between the COMMIT intervention and comparison communities.
- 3) Evaluation Cohort Survey—Cohorts of 400 adults from each of the 22 communities were contacted a third and final time in the spring of 1993 to measure the population-wide impact of COMMIT on intervention awareness, participation, and the decline of the social acceptability of smoking.
- 4) Final Prevalence Survey—A cross-sectional sample of 3000 adults in each COMMIT community were surveyed to determine adult smoking prevalence. The survey was conducted between August 1993 and January 1994 and is providing a measure of the trial's secondary endpoint, smoking prevalence.

The Branch has planned the statistical analyses for the major COMMIT endpoints. Other work has involved approaches for the weighting and adjustment of results from the prevalence surveys, with consideration of probability of inclusion of each telephone number in the sampling frame, number of telephones in each household, nonresponse, and age-sex distribution for each community based on 1990 census data.

The major effort this year has been the analysis of the major endpoints and preparation of the two major publications reporting the outcome.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CN 00197-01 BB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Brain Tumor Clinical Trials

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: S.B. Green Lead Research Investigator CDTs, BB, DCPC, NCI

COOPERATING UNITS (if any)

Information Management Services, Inc.
German Cancer Research Center

LAB/BRANCH

Biometry Branch, OD, DCPC

SECTION

Clinical and Diagnostic Trials Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.3

PROFESSIONAL:

0.3

OTHER:

0.0

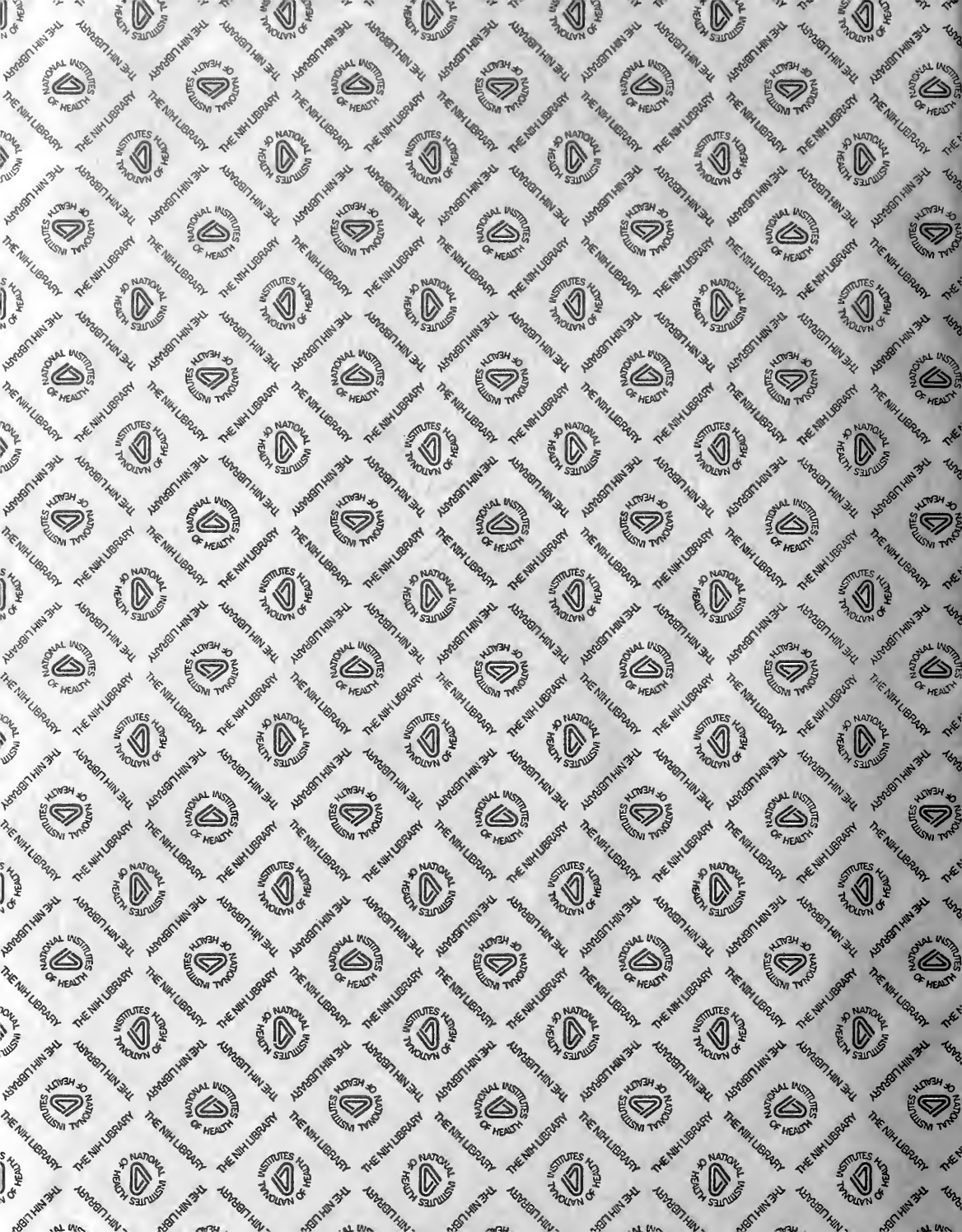
CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human Subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The Clinical and Diagnostic Trials Section provides full statistical support for the Brain Tumor Cooperative Group (BTCG), a multicenter group of neurosurgeons, neuro-oncologists, radiotherapists, neuro-radiologists, and neuro-pathologists conducting randomized trials for patients with primary brain tumors (with emphasis on malignant gliomas). During this past year, the BTCG completed the accrual of patients to a randomized Phase III trial, BTCG 87-01, investigating interstitial radiation (seed implants) as an addition to the customary external beam radiation and chemotherapy. Preliminary analyses of the data have been performed and the results presented. Patients randomized to interstitial radiation had increased survival compared to the standard arm; this difference was statistically significant in both the Randomized Population and the Valid Study Group. As with past studies, age, Karnofsky performance status, and histopathology were found to be significant prognostic factors. Models adjusting for the three prognostic factors suggested that the treatment difference in favor of interstitial radiation occurred for Glioblastoma Multiforme but not for the other malignant gliomas. (However, this latter subgroup had small numbers and differences were not statistically significant.) Pathology review at time of surgery following possible "failure" showed that categorization by viable tumor versus necrosis (with or without tumor cells) was significantly predictive of subsequent survival.

During this year, accrual was completed on another randomized trial, BTCG 89-01, that compares two Phase III chemotherapy regimens to be given in addition to surgery and radiotherapy. One regimen is the standard intravenous BCNU; the second is the combination of intravenous BCNU with intra-arterial cisplatin. The trial had also included a third arm in the randomization, used to investigate, successively, new investigational Phase II drugs. The initial agent was 10-EDAM (Edatrexate). When accrual for this group was completed in early 1992, randomization was begun to Piroxantrone. Accrual to this third arm was later terminated early by the Division of Cancer Treatment (who funds the BTCG and is responsible for all official NCI decision-making concerning these trials), because of a decision not to pursue the agent Piroxantrone. Followup continues on all patients on BTCG 89-01, with appropriate data monitoring.





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